CHARACTERISATION AND SPECIATION OF FUNGAL ISOLATES IN NEONATAL SEPSIS IN A TERTIARY CARE HOSPITAL

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BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled **"CHARACTERISATION AND SPECIATION OF FUNGAL ISOLATES IN NEONATAL SEPSIS IN A TERTIARY CARE HOSPITAL"** submitted by **Dr. V. LAKSHMANA KUMAR** to the Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the requirement for the award of M.D degree Branch– IV (Microbiology) is a bonafide research work carried out by him under direct supervision & guidance.

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DECLARATION

I, **Dr. V.LAKSHMANA KUMAR** declare that, I carried out this work on, **"CHARACTERISATION AND SPECIATION OF FUNGAL ISOLATES IN NEONATAL SEPSIS IN A TERTIARY CARE HOSPITAL"** at the Institute of Microbiology, Madurai Medical College. I also declare that this bonafide work or a part of this work was not submitted by me or any others for any award, degree or diploma to any other University, Board, either in India or abroad.

This is submitted to The Tamilnadu Dr. M. G. R. Medical University, Chennai in partial fulfillment of the rules and regulations for the M.D. Degree examination in Microbiology.

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CONTENTS

CHARACTERISATION AND SPECIATION OF FUNGAL ISOLATES IN NEONATAL SEPSIS IN A TERTIARY CARE HOSPITAL.

Abstract

Introduction: Sepsis is the most common cause of neonatal mortality. Sepsis caused by fungal infections are a serious problem. This study is aimed to estimate the incidence of fungal sepsis, evaluate the risk factors and isolate the common fungal pathogen in neonatal intensive care unit(NICU) in a tertiary care hospital.

Materials and methods: 200 neonates were selected from NICU and blood, CSF or urine sample were collected for fungal culture. Fungal pathogens were isolated by conventional culture method, identification by microscopic and macroscopic observation of growth. Speciation of Candida was done by germ tube test, corn meal agar morphology, CHROMEagar color, urea hydrolysis, sugar fermentation and sugar assimilation tests. Antifungal susceptibility testing was done by broth dilution, agar dilution and disc diffusion methods.

Results: From 200 samples, 13 fungal growth were isolated. The incidence of fungal sepsis was 6.5%. All the isolates were Candida species and non albicans Candida were predominent (77%). C.albicans (23%) and C.gullermondii (23%) were the most common species. There was male preponderance (54%) and outborn babies (62%) had more sepsis. The major risk factors were preterm (77%) and very low birth weight (VLBW) (69%) and the associated risk factors were prolonged hospital stay more than 7 days (54%), administration of broad spectrum antibiotics for more than 5 days (61%) and mechanical ventilation (46%). The isolated candida species showed more resistance to fluconazole (30%) and amphotericin B (23%).

Conclusions: The incidence of fungal sepsis in NICU was increased. Candida species were the common cause for fungal infection in neonates. Preterm and very low birth weight, prolong hospital stay, administration of broad spectrum antibiotics and mechanical ventilation were the major risk factors. Nosocomial infection was considered as a major contributor. Routine screening and evaluation can be recommended for prevention and control of fungal sepsis in NICU.

Key words: NICU, fungal sepsis, VLBW neonates, Candida species.

INTRODUCTION

Microorganisms infect humans by various routes like skin contact, respiratory system, gastrointestinal system, genitourinary system, direct spread through blood or transplacental transmission. In invasive infections the microorganism circulates through vascular system and affect any organ system of human body. Sepsis is a clinical syndrome which results in circulating microorganism which multiplies at a rate that exceeds their removal by phagocytes. The symptoms are produced by microbial toxins and cytokines from inflammatory cells.[70]

Definition of sepsis:

'Sepsis' is defined as systemic illness caused by microbial invasion of normally sterile parts of the body and systemic inflammatory (SIRS) response syndrome due to the invasion. This term differentiate an illness with identical clinical syndrome that can arise in several non-microbial conditions. SIRS defined as presence of at least two of the following signs like temperature above 38 ℃ or below36℃, tachycardia, increased respiratory rate or PCO2 below32 Torr, leukocytes above 12000/µl or below4000/µl or above 10 % of band cells. This becomes 'severe sepsis' when there is evidence of hypoperfusion or dysfunction of

at least one organ system. When there is persistence of hypotension in spite of fluid resuscitation or need for vasopressors ,it is termed as 'septic shock'.[47,74]

Neonatal infection:

Neonatal infections are unique in several ways. Infectious agents can be transmitted from mother to fetus or community acquired or may be nosocomial. Newborn infants are less capable of responding to infections and coexisting condition often complicate the diagnosis and management. The clinical manifestation is varying from subclinical to severe. There may be focal or systemic infection and rarely, congenital syndromes resulting from in utero infection. The etiologic agents are wide variety, including bacteria, viruses, fungi, protozoa and mycoplasma. Preterm, very low birth weight (VLBW) babies need a prolonged hospital stay which puts them at continuous risk for acquired infections.[13]

Predisposing factors:

A newborn is deficient in various components of humoral and cellular immunity. The chemotaxis, phagocytosis, bactericidal activity and cytokine production are reduced due to immature polymorphonuclear and macrophages activity. Immunoglobulin and complement system are also immature, more so in premature neonates.[140]

Hospital Acquired Infection (HAI)

The term HAI (syn; Nosocomial) is applied to any infection causing illness that was not present or in its incubation period when patient entered hospital or received treatment in an outpatient or accident and emergency department.[78]

HAI in neonates:

The Centers for Disease Control and Prevention (CDC) defines HAI in neonates as any infection that occur after admission to NICU and that was not transplacentally acquired. It is also defined as any infection occurring more than 5 to 7 days after birth in a hospital delivered babies.

Impact of HAI in newborn care:

Rates of HAI in well maintained newborn nurseries are low, ranging from 0.3 to 1.7 per 100 newborns. Rates of healthcare-associated neonatal infections are 3 to 20 times higher in resource limited countries. Low birth weight is a major risk factor for HAI with each 100g decrease in birth weight conferring an additional 9% risk of BSI. Infection rate is increased in NICU due to overcrowding, invasive procedures, more exposure to infectious agents and contamination of environment.[16]

Importance of newborn care:

Newborn health is the key marker of child health. Above 50% of under five child deaths occur during newborn period. In that, most of newborn deaths (40%) occur within 24hrs.[139]

Newborn death is decreased due to improved maternal care, well established delivery system and advancement in neonatal care. But newborn deaths are still accounted for higher share of global child death. Most of the deaths happen in just five large countries-India, Nigeria, Pakistan, China and Democratic Republic of Congo. Three major causes of neonatal death in the world are preterm delivery (29%), asphyxia (23%), and severe infection (25%).[59]

Infant mortality rate (IMR):

Infant mortality rate (IMR) is defined as the ratio of infant deaths that are registered in a given year to the total number of live births registered in the same year, usually expressed as a rate per 1000 live birth. Neonatal death is the death that occur during the neonatal period starting from birth to 28 days of life.[96]

Neonatal mortality ratio (NMR):

Neonatal mortality ratio (NMR) is the number of neonatal death in a given year per 1000 live birth in that year.[96]

Incidence in India:

India accounts for 20% of global births and 30% of global newborn deaths. Infections (33%), asphyxia (21%) and prematurity (15%) are the leading causes of neonatal deaths in India. Most of this death can be prevented in various levels such as awareness in community level, maternal health care and new born care.[99]

In India under five mortality in 2012 was 56, IMR was 44 and NMR was 31 per 1000 live birth. IMR in Tamil Nadu was 21 per 1000 live birth.[40]

Neonatal sepsis:

The clinical spectrum of neonatal sepsis begins with a localized infections (like meningitis, pneumonia and pyelonephrits) or from systemic infections (by bacteremia, fungemia and viremia). Then there is systemic inflammatory response syndrome (SIRS), which may progress to severe sepsis (sepsis combined with organ dysfunction), septic shock (persistence of hypoperfusion or hypotension), multiple organ dysfunction syndrome (MODS) and even death. Initial presentation may be limited to only one system, but involvement of another system should also be evaluated.[91]

Classification of neonatal sepsis:

Neonatal sepsis can be classified into two major categories according to the time of onset

Early onset of sepsis (EOS) presents within the first 72 hours of life. In severe cases, the neonate may be symptomatic at birth. Infants with EOS usually present with respiratory distress and pneumonia. The source of infection is generally, the maternal genital tract. Some maternal / perinatal conditions have been associated with an increased risk of EOS. Knowledge about this potential risk factor would help in early diagnosis of sepsis. The following factors such as low birth weight and prematurity, intrapartum sepsis, rupture of membranes for more than 24 hrs, prolonged labor, perinatal asphyxia and foul smelling and meconium stained liquor seem to be associated with an increased risk for early onset sepsis.[39]

Late Onset Sepsis (LOS) presents after 72 hours of age. The source of infection in LOS is either nosocomial or community acquired and neonates usually present with septicemia, pneumonia or meningitis. Various factors that predispose to it which includes low birth weight, prematurity, admission in intensive care unit, mechanical ventilation, invasive procedures, administration of parentral fluids, and usage of stock solutions. Factors that might increase the risk of community acquired LOS include poor hygiene, poor cord care, bottle feeding, and prelactal feeds. In contrast, breastfeeding helps in prevention of infections.[39]

Aspiration of infected amniotic fluid may produce pneumonia and sepsis in utero, manifested by fetal distress or neonatal asphyxia. Exposure to pathogens during delivery and in the nursery care or community is the mechanism of

infection after birth. The physiological manifestations of the inflammatory response are mediated by a variety of proinflammatory cytokines, principally Tumor necrosis factor (TNF), Inter leukin - 1 (IL-1) and IL-6 and byproducts of activation of the complement and coagulation systems. Elevated levels of IL-6, TNF and Platelet activating factor have been reported in new born infants with neonatal sepsis. IL-6 is the cyokine most often elevated in neonatal sepsis.[88]

Etiological agents:

The common etiological agents for EOS are Group B Streptococcus(GBS), Escherichia coli(E.coli), Streptococcus viridians, Enterococcus species, Staphylococcus aureus (S.aureus), group D Streptococcus, Pseudomonas and other Gram negative enteric bacilli. In LOS the common organisms are coagulase Negative Staphylococcus (CoNS), S.aureus, E.coli, Klebsiella spp, Candida spp, Enterococcus spp, Pseudomonas and group B Streptococcus(GBS).[89]

Outbreaks of viral infection in NICU can be serious and difficult to control. Enteroviruses such as echovirus 11, adenovirus 7 & 8, rotaviruses and respiratory syncytial virus(RSV) may be responsible for most cases.[79]

Clinical presentation of sepsis:

The earliest signs of sepsis are often subtle and nonspecific; indeed a high index of suspicion is needed for early diagnosis. Neonates with sepsis may present

with one or more of the following symptoms and signs such as hyper/hypothermia, fever, lethargy, poor cry, refusal to suck, poor perfusion, Hypotonic, absent neonatal reflexes, bradycardia or tachycardia, respiratory distress, apnoea and gasping respiration, hypoglycemia or hyperglycemia and Metabolic acidosis.[64]

Fungal infection:

Fungal infections are common and some of them are serious and even fatal. Most bacterial infections have been managed well, but fungal infection has not been so. Most fungi are saprophytes in the soil and human mycosis infections also cause as many fatalities as whooping cough, diphtheria, scarlet fever, typhoid, dysentery and malaria.[4]

Apart from bacterial pathogens, fungi also causing systemic infections and sepsis in NICU. This fungal sepsis is mostly nosocomial and known hazard of prolong life support techniques like ventilator care, venous catheterization, exchange blood transfusions and total parentral nutrition(TPN), prolonged antibiotic administration for bacterial sepsis.[83]

Fungal pathogens in neonatal infection:

Fungi are ubiquitous environmental organisms, also frequently found in hospital environment. Fungal infections are common in neonates and presented as a trivial mucocutaneous infection to life threatening fungemia and deeply invasive

mycosis. Among the fungal pathogens, Candida species is the dominant one, and there is equal distribution between C.albicans and non albicans candida(NAC) species. Other fungi isolated in neonatal infections are Aspergillus fumigatus, A.flavus, Malassezia furfur, M. pachydermatis. From invasive fungal dermatitis, apart from Candida species and Aspergillus species, Tricosporon beigeli and Curvularia species are rarely isolated. Candida species are the third most common cause of LOS in the NICU, but the mortality is sevenfold higher than Staphylococcus epidermidis.[85]

Pathogenesis of fungal infection:

The pathogenesis of fungal infections involves adherence, colonization, infection and dissemination. Surface glycoprotein help in fungal adherence. In yeast cells, surface glycoprotein-INT1 binds to beta- integrins present on the endothelium and WBCs. Filamentous form of fungus have increased virulence in immunocompromised patients.[26]

Aspergillosis generally follows inhalation of spores but also enter directly into wounds or during surgery. It is presented as allergic form, non invasive colonization and invasive Aspergillosis. Invasive Aspergillosis is mainly chronic necrotizing pulmonary and also disseminated to any organ system of human body.[15]

Malassezia yeasts interfere with melanin production in skin. It requires free fatty acids for survival. TPN with lipid hyperalimentation leads to entry trough intravenous line. The usual manifestation is lipid deposits in pulmonary arteries.[8]

Zygomycosis infection can occur by inhalation, percutaneous inoculation or ingestion. They have a predilection for elastic lamina of large and small arteries causing thrombosis, hemorrhage and infarction.[57]

Immunity against fungal infection:

Fungi are eukaryotic organisms and its protein synthesis machinery and mechanisms to organize and replication of the genome are similar to mammalian cells. Phagocytes particularly neutrophils and macrophages are essential for killing fungi either by degranulation and release of toxic materials in to hyphea or ingestion of yeast or conidia. Most fungi are highly immunogenic and induce strong antibody response and T cell mediated immune response. Considerable evidence proves that dominant protective role of TH 1 and phagocyte activation is important than antibody mediated response. Fungi possess many evasion strategies to promote their survival. In Candida species, concealing the beta Glucans of their cell wall underneath a external coat of Mannan, it is considerably less immuno reactive.[43]

Risk factors for fungal infection:

Risk Factors for Invasive Fungal infection(IFI) in neonates are gestational age < 32 weeks, birth weight ≤ 1500g, male gender, APGAR score below 5 at 5 minutes, intubation and mechanical ventilation, placement of indwelling devices, umbilical catheters, peripheral or central venous catheters, urinary catheters, cerebrospinal fluid shunt devices, abdominal surgery, lack of eternal feeding, use of intralipid, total parenteral nutrition(TPN) supplementation, corticosteroid use, use of histamine type 2 receptor blockers and prolonged antibiotic administration.[33]

Candida infection:

Neonates are a special population at risk of Candidemia and disseminated candidiasis. For some 2000 years after Hippocrates described thrush in the mouths of babies, the infection was viewed as an annoying and insignificant superficial disease. Only within the past century, Candidiasis of deep organs has been recognized. Since the 1960s, certain important therapeutic and diagnostic advances in medicine have affected host defenses and microbial ecology to the extent that these ubiquitous yeasts have gained prominence as pathogens of the first order with capabilities of producing life-threatening disease.[63]

Candida exists in three different morphological forms. Yeast cells or blastospores have oval to round in shape, double walled chlamydospores usually located at the end of a pseudohypha and pseuodomycelium- a mass of

pseudohypha mostly in tissues. [104]

It manifests as congenital cutaneous candidiasis, mucocutaneous candidiasis, urinary tract infection, blood stream infection and disseminated infection. C.albicans is the most frequently isolated yeast species in the specimen taken from infected neonates. Neonates and pregnant women have impaired host resistance to Candida species. Advances in health care have decreased the mortality for many conditions also have been associated with changes in host defense and normal flora, which have in turn led to a large population at risk for invasive Candida infection.[66]

Non albican Candida:

Although Candida albicans remains the most common fungal pathogen isolated from blood and body tissue, recent literature have shown an increased prevalence of non albican Candida (NAC). NAC (c.parapsilosis, c.tropicalis, c.glabrata) exhibits varying levels of resistance to flucanazole and other antifungal agents .Small percentage of infection are due to C.lusitaniae, C.gulliermondii, C.dubliniensis.[58]

Disseminated Candidiasis:

Disseminated candidiasis may present with several entities like meningitis, endocarditis, renal abscess, endoptholmitis, CNS absecess, ventriculitis and liver

absecess. It is recognized, when infant fails to respond antibiotics or when these infants have unexplained feeding intolerance, Cardio vascular instability, apnea, a new (or) worsening of respiratory failure, increasing ventilation requirement, lethargy, convulsions and poor activity. There may be thrombocytopenia, hyperbilurubinemia, elevated C-reactive protein (CRP) and glucose instability (hypoglycemia or hyperglycemia).[114]

Malassezia infection:

Malassezia species is dimorphic lipophilic yeast. Malassezia furfur is isolated from neonates and has been associated with fungemia and occasionally pneumonia in premature infants with low birth weight who are receiving liquid emulsion alimentation through central venous catheters. Sporadic bloodstream infections have been described, with transmission probably by the hands of medical personnel. Malassezia pachydermatis has also reported in VLBW infants with sepsis.[129]

Aspergillus infection:

Invasive infections with filamentous fungi are rare, but sporadic cases reported. Aspergillosis is less common than invasive Candidiasis in newborn and is usually a primary cutaneous disease. Aspergillus fumigatus is the most common

species followed by A. flavus in invasive Aspergillosis. Prematurity is one of the main risk factor. The lung is the most commonly affected organ.[129]

Zygomycosis:

Zygomycosis, a filamentous fungi infection usually start at a site of local trauma or intravenous catheter insertion site or infiltrate and progress to a necrotizing soft tissue infection. Most of it presented as gastrointestinal infection and some mimics Necrotizing EnteroColitis (NEC). Early diagnosis and treatment needed to prevent rapid fatal dissemination. The common species are Rhizopus, Mucor and Rhizomucor.[35]

Tricosporon infection:

Tricosporon beigelii rarely isolated from invasive fungal dermatitis. It leads to systemic complication like endocarditis, brain abscess, pulmonary infections and endophthalmitis.[35]

Admission criteria for newborn:

The admission criteria for neonatal intensive care unit (NICU) are birth weight <1800g or gestation <34 week, large baby >4.0kg ,refusal of feeds, respiratory distress(rate >60/minute, grunt, retractions), severe jaundice, hypothermia <35.4℃ , hyperthermia >37.5℃, central cyanosis, shock (cold

periphery, CRT >3sec), coma, convulsions, encephalopathy, abdominal distension, diarrhea, dysentery and major malformation, These newborn babies have features of sepsis or more prone for sepsis.[97]

Diagnosis of fungal infection:

A high index of suspicion and early diagnosis is needed to reduce the morbidity and mortality. Isolation of the causative agents from a relevant clinical specimen, screening for hematologic markers, serological tests and molecular studies are useful for diagnosis of fungal infections.

Septic screen:

All neonates suspected to have sepsis should have a septic screen to correlate the diagnosis. The various components of the septic screen include total leukocyte count, absolute neutrophil count, ratio of immature neutrophil to total neutrophil count(I/T ratio), micro-erythrocyte sedimentation rate(micro-ESR) and C reactive protein(CRP). The absolute neutrophil count varies considerably in the immediate neonatal period.[109]

Definitive diagnosis:

Isolation of organism by fungal culture from various samples like blood, CSF and urine is the standard test. Speciation done by observation of culture

growth morphology, microscopic structures and specific confirmatory biochemical tests. Antifungal susceptibility is mandatory now a days to guide the clinician for the treatment and to prevent the drug resistance.[86]

Serological tests:

Fungal serology tests are tube precipitation, immunodiffusion, latex agglutination and complement fixation. Enzyme immunoassay with species specific monoclonal antibody, nucleic acid probe assay and polymerase chain reaction (PCR) also used in some laboratories. Fungal antigens that used in rapid diagnostic tests are Galactomannan in invasive Aspergillosis and Beta-D glucan, mannan in candida infection.[69]

Monitoring:

In a suspected or proved cases of Candidiasis, additional tests like complete blood count (CBC), blood urea nitrogen, creatinine, potassium and liver enzymes should be done. End organ dissemination should be screened by Echocardiography, Renal ultrasonography, neurosonogram and indirect ophthalmoscopy for every newborn.[76]

Management:

Prompt initiation of systemic antifungal therapy, removal or reduce the

exposure of indwelling catheters, minimize the contamination and supportive care are needed to prevent dissemination and improve outcome.

Supportive care:

Adequate and proper supportive care is crucial in a sick neonate with sepsis. This cases should be nursed in a thermo-neutral environment taking care to avoid hypo or hyperthermia. Oxygen saturation should be maintained in the normal range, mechanical ventilation may have to be initiated if necessary. If the infant is hemodynamically unstable, intravenous fluids should be administered.[143]

Antifungal therapy:

There are some guidelines outlining the choices for optimal therapy in the treatment of neonatal fungal infection. Amphotericin B deoxycholate, a polyene antifungal drug with broad spectrum of activity is the mainstay of therapy. Amphotericin B deoxycholate is better tolerated in neonates. Lipid formulation of Amphotericin B reduces the nephrotoxicity and infusion related toxicity. C.lusitaniae is resistant to Ampotericin. Flucytosine (5-FU), a fluorine analog of cytosine , mostly used in combination with Amphotericin B.[42]

Flucanazole is a fungistatic that inhibit the production of ergosterol in fungal cell membrane. It is the only agent used in neonatal populations with significant research data. Fluconazole is inactive against all strains of C.krusei and

some of C.glabrata and also not useful against Aspergillosis, Zygomycosis. Another azole Voriconazole is active against C.kruesi, C,glabrata and Aspergillosis but not effective for zygomycetes.[42]

Echinocandins, a newer class which target the cell wall by inhibition of (1, 3)-Beta-D-glucan synthase complex. Caspofungin is approved to treat Aspergillosis and Candida infection in neonates. Studies are underway to determine the effectiveness of this drug in neonatal patients.[42]

Prophylaxis:

Fluconazole is used for prophylaxis in many NICUs to reduce the fungal colonization and prevent the invasive infection among VLBW babies. It is advised as dose of 3mg/kg twice weekly and continued up to 6 weeks. Newborns in NICU who receive prolonged antibiotic therapy and on CVC are also benefited by prophylactic Fluconozole.[77]

Nystatin, a polyene antifungal used topically and orally to prevent colonization over skin, mucus membrane and gastrointestinal tract. It is safe, inexpensive and well tolerated drug.[38]

Prevention:

Principles for the prevention of nosocomial infection include adherence to universal precautions, avoiding nursery crowding, strict compliance with hand

washing, meticulous neonatal skin care, minimizing the risk of catheter contamination, decreasing the number of veni-punctures and mechanical ventilation days and providing education and feedback to nursery personnel. Surveillance for HAI is essential for prevention and control of sepsis. Infrastructure designing with adequate space and equipments, regular decontamination and cleaning of nursery, sufficient staffing in NICU and screening, periodic health check up, vaccination for employee are all helpful measures in prevention.[71]

Apart from diagnosis and treatment of bacterial sepsis in NICU, fungal infections and sepsis are also an important problem. There are gaps in understanding and there is an urgent need for studies about simple and sustainable intervention to reduce the burden of neonatal sepsis. To reduce the morbidity and mortality by fungal infection, a better understanding of the incidence, diagnosis, management, definite therapy and prophylaxis is essential. Assessment of risk factor, clinical features and investigation profile and isolation of fungal pathogen by this study will help in early intervention with antifungal treatment. It will also leads to a good clinical outcome in neonatal sepsis cases, decrease the morbidity and mortality among these cases, decrease the duration of hospital stay and treatment cost in NICU.

AIM AND OBJECTIVES

Aims and Objective

Aims and Objective are:-

1. To study the Prevalence of neonatal fungal sepsis.

2. To identify the risk factors for development of fungal sepsis in neonates.

3. Characterization and speciation of the fungal isolates and test the antifungal drugs susceptibility pattern.

4. To guide the clinician for the appropriate treatment with antifungal drugs and help to improve the clinical outcome.

5. To give the guidelines in controlling fungal infection in the NICU.

REVIEW OF LITERATURE

Review of Literature

Neonatal sepsis

Stoll BJ (1997), has documented in his article 'The Global Impact of Neonatal Infection', sepsis is the commonest cause of neonatal mortality and it is responsible for 30-50% of the total neonatal deaths in developing countries.[131]This was also reported by **Agrawal et al,** (2001).[2]

Vergnano et al,(2005) reported incidence of neonatal sepsis varied from 7.1 to 38 per 1000 live births in Asia, 6.5 to 23 per 1000 live births in Africa, and 3.5 to 8.9 per 1000 live births in South America.[138]

A study in Nigeria by **U. Edewochi et al**,(2012) stated that perinatal asphyxia, LBW , neonatal sepsis and neonatal jaundice were leading cause of admission in NICU. In out born babies delay in seeking care and diagnosis was significantly affecting the outcome.[33]

Neonatal sepsis is predominant among male infants. Term male infants have an approximately twofold higher incidence of sepsis than term female as stated by **Washburn et al,** (1969).[142]

Neeraj kumar et al, (2010) stated that 72% male neonates were affected by neonatal sepsis in their study.[93] The gene located at X chromosome is involved with the function of thymus or with the synthesis of immunoglobulin. Double X

chromosome in female gender leads a greater resistance to infection. This was explained by **Bellani AJ, Schlegel,** (1969), in the textbook of Infectious Disease of the Fetus and Newborn.[14]

Cloherty J P and Strak R, (1998) classified neonatal sepsis into two major categories depending upon the onset of symptoms. Early onset sepsis presents within 72 hrs of age and late onset sepsis presents after 72 hrs of age. [23]

Franciosi et al, (1973) observed generalized disease takes two clinically and epidemiologically distinct forms, early and late onset. By comparison, United States and Australia 1.5 to 3.5 per 1000 live births affected by EOS and 6 to 9 per 1000 live births by LOS.[36]

Reports from India showed 50-60% of septic babies were premature babies and very low birth weight babies. This was documented by **Bang et** al, (2001).[12] Based on study from India by **Singh** et al,(1994) identified the risk factors associated with early onset sepsis.[127] **Sundarm et al**,(2009) studied the incidence of neonatal sepsis, it was found that the incidence of LOS increased from 12 to 16.5 per 1000 live births and the incidence of EOS remains at 3.5 to 8.9 per 1000 live births.[133]

Kanya Mukuhopadhyay et al,(2012) reported that major causes of mortality in ELBW neonates are sepsis (46%). birth asphyxia (20%) and pulmonary hemorrhage (19%).[62]

The increased incidence of septicemia among premature neonates was attributed to their poor immune response like low level of IgG, impaired cellular immunity and poor immune response and poor mucosal defense according to **Schreiber** et al (1992).[120]

Haque K H (1988) has defined 'Neonatal Septicemia' is a clinical syndrome characterized by systemic signs and symptoms in the first month of life. It encompasses systemic infections of newborn including meningitis, pneumonia, arthritis, osteomyelitis and urinary tract infection of the new born.[46]

HAI

In a study by **Sengupta, et al**(2010), the incidence of catheter related BSI for CVCs or PVCs were increased in first three weeks, but if indwelling duration was above 35 days there were more risk (33%/day). Umbilical catheter for >4 days was found to increase the risk of sepsis dramatically.[105]

In their study **Auriti C et al**,(2003) suggested that approximately one third of nosocomial infections had been prevented by effective infection control protocols. Despite surveillance and preventive measures, the risk was higher in NICU.[9]

Late-onset-sepsis is either nosocomial (hospital-acquired) or community acquired and neonates usually present with septicemia, pneumonia or meningitis according to **Wolach B** (1977).[145]

In developing countries the incidence of nosocomial infection was studied by **Zaid et al,(**2005) and it varied between 18% and 34% and it accounted for as much as 40% deaths in neonatal units. [149]

In a Brazil study about incidence of nosocomial infection by **Karla Dal-Bo et al,** (2012) reported about 33% mortality in NICU, which was equal to any other developing countries. There was 78% of bloodstream infection noted in that study. [65]

In a national point prevalence study in US by **Sohn AH et al,** (2001) reported nosocomial infection rate of 11.4%. The major interventions noted among those infections were CVP associated 23.3%, ventilator associated 22.9% and TPN associated 23%.[128]

In Taiwan, **Bai-Horng, et al**,(2007) studied about the distribution of pathogens for nosocomial infections in NICUs and there was 10.5% of fungal pathogens in bloodstream infections.[10]

Surveillance and Control Pathogens of Epidemiological Importance **(SCOPE)** investigators analyzed about HAI-BSI outcome of 49 medical centers in US and noted a crude mortality rate of 27% and 40% for candida spp. during 1995 to 2004.[144]

Fungal sepsis:

Paolo Manzoni (2013) stated that, fungi were the third most common agent of late on set sepsis (LOS) in preterm neonates. Invasive fungal infection (IFI) was usually preceded by its colonization. Colonized CVC was associated with tenfold high risk for progression of sepsis. Prophylactic Flucanazole greatly reduced the risk and oral Nystatin are also effective.[94]

Changing Pattern of neonatal fungal sepsis in a developing country Studied by **Daynia E. Ballot et al** (2013) in South Africa and reported the incidence of fungal BSI in neonates increased over time (0.6% in 2007 to 1.8% in 2011).[27]

Richard A, et al, (2003) tabled the infectious pathogens in NICU. Among the fungal pathogens, Candida species were the third most common isolates in blood stream infections and sepsis and Malassezia species were also isolated less commonly in TPN supplemented newborns . Candida species also isolated from urinary tract infections, endocarditis, CNS infections and osteoarthritis. Aspergillus species isolated from skin, soft tissue or surgical site infections and it was categorized as less common one.[107]

The incidence of systemic fungal infection in newborn had been observed by **Stoll BJ**, et al (1996) , which ranged from 2.2% to 12.9% among VLBW babies and from 3.5% to 16.3% among ELBW babies**.**[130]

A prospective study was conducted by **Rolides et al** on invasive candidiasis in the NICU of Aristotle University in Hippokration hospital (1994-2000), observed that overall incidence of 1.28% and overall mortality for C.albicans is more (39.5%) than C.parapsilosis (11.1%).[111]

A prospective population based study for candidamia in Spain by **Rodriguez D, et al,** (2002-2003),resulted with an annual incidence of candidamia 32.6 cases per 100,000 live births and 1.1 cases per 100 discharged cases from NICU.[110]

A surveillance study about fungal colonization by **Tushar BP et al (**2007), showed significant decrease in fungal colonization by Fluconazole prophylaxis for the first 28 days period in extreme premature neonates with birth weight below 1000 grams and gestational age at birth below 30 weeks. Non albicans candida become a frequent fungal species in colonization and infection in NICU especially in VLBW neonates.[134]

Clerihew et al, (2005) estimated the incidence of invasive fungal infection (IFI) was 10 cases 1000 per live born VLBW infants in a national prospective surveillance study at UK. They isolated fungal pathogen from 92 VLBW infants, and most of them were candida species (87/92). There were two Malassezia species, one Aspergillus species, one Rhizopus species and one unidentified yeast

also isolated. The mortality among above was 40% due to invasive fungal infection.[20]

In North India **Singh K** et al,(1999) reported incidence of 22.8% of invasive fungal infection in preterm babies who stayed for more than one week in NICU.[126]

VP Baradkar et al (2008), reported a case of neonatal septicemia due to Candida dubliniensis in a premature infant. C.dubliniensis is a part of normal flora of gut. But it is an emerging pathogen that can cause invasive disease. [141]

Rabindra N. Misra et al (2013) isolated one Candida species, C.parasilopsis among 75 blood culture positive neonatal sepsis cases in a screening study at a NICU, tertiary care center in Pune. CRP was positive for 68 cases of positive blood culture newborns.[103]

Procinary RS, et al (2007), documented that fungal sepsis was more common in the severe premature infants, newborns under mechanical ventilation support, long term parenteral nutrition, with hypoglycemia and exposed to multiple antibiotic therapy. Candida species were the major pathogens for fungal sepsis and transmitted from maternal flora or from nosocomial infection. The mortality rate was 45.8% and the majority of affected neonates had been associated with risk factors like assisted ventilation, mechanical ventilation, nasal continuous positive airway pressure(CPAP) ventilation. There was high prevalence of surgical

problems (44%) like necrotizing enterocolitis among the fungal sepsis cases. Abdominal Surgery was also a risk factor for fungal sepsis in neonates.[102]

Daniel K. Benjamin et al (2010) reported that invasive candidiasis increased the risk of death. It was highest among the neonates whom candida isolation from multiple sources. (eg urine and blood (or) urine and CSF).Nearly 60% of infants with invasive candidiasis died.[25]

Baley et al(1991) study was showed that Candida species rapidly colonize the skin and mucus membrane of about 40-60% in critically ill infants and can progress to invasive infection. Usage of Fluconazole prophylaxis in extreme premature newborns, BW <1000grams and gestational age below 27 weeks had been proved to a good option for prevention of fungal sepsis .Resistant to Fluconazole was also reported for some of the cases. So prophylactic use of flucanazole needs caution.[11]

In Brazil, three cases of C. krusei were reported in a study of neonatal fungal sepsis by **Selma et al** (2012). It had been recognized as a potentially multidrug resistant fungal pathogen with intrinsic resistant to Flucanazole and decreased susceptibility to amphotericin B. Resistance pattern of C.parapsilosis was more for Fluconazole(5.4%) but voriconazole resistant also noted in some isolates.[122]

Candida :

Candidiasis is a "white plague" of the immunocompromised host. Clinical expression of the disease implies debility, ranging in magnitude from the weak neonate to the individual with a profound congenital or acquired immunodeficiency disorder. Exposure via horizontal and vertical transmission, from vaginal candidiasis in pregnant mothers and hand colonization of care givers. A study about annual incidence of neonatal candidemia conducted during 1995-2004, and the attack rate of candida (Blood stream infections per 100 patients) and incidence density (BSIs per 1000 days) was determined. Among the ELBW neonates the incidence was decreased from 3.51 (1995 to 1999) to 2.68 (2000-2004). There were 1997 cases of BSI's, most common organism was Candida albicans and also C.tropicals, C.lusitaniae, C.glabrata and C.krusei were also isolated. This study justifies the fluconazole prophylaxis for candidial colonization.[121]

Vandhana Sardana, et al (2012) reported candidemia in 30% of cases of neonatal sepsis and the NAC species were predominant in their study. There was a changing trend in neonatal candidemia.[136]

Rippon JW (1998) described about iatrogenic and barrier break Candidiasis that was resulted from wide variety of insults to the neonates who were admitted in intensive care. Colonization and invasion was occurred in association with

indwelling catheters, hyperalimentation and surgical procedure or simply through intramuscular or intravenous injections and during lumbar puncture procedure that spreads directly to central nervous system(CNS).[108]

Ray TL (1989) described an additional aspect of Candida, that there was a capacity to undergo high frequency "phenotype switching" within single strain, which probably contributes to the pathogenicity of some strains,. That phenotypic variability were represented in an individual cell or yeast colony, drug sensitivities and secretion of protective virulence factors like acid proteinase. Rapid differential gene expression by the organism might allow important pathogenic adaptability of the organism to the host environment.[109]

Malassezia pachydermatis, an animal pathogen, can also colonize infants in the NICU and cause fungemia in association with intravenous administration of lipids. Invasive fungal dermatitis is an entity that is recognized increasingly with the infants with VLBW. These infants have the same risk factors for invasive fungal infection as described for neonatal candidiasis. Invasive infections with filamentous fungi are rare in the newborn, but sporadic cases of aspergillosis and zygomycosis have been reported. Aspergillus infections in neonates present as cutaneous or disseminated disease. Gastrointestinal infections account for more than 50% of reported cases of zygomycosis in neonates; some cases mimic NEC. Extreme prematurity, acidosis, renal failure, and treatement

with steroids are risk factors for invasive mold infections. Mortality rates are high, and diagnosis is often made only at autopsy.[16]

Zygomycosis was reported by **Roildes E** et al, (2009)that >70% of the infection were in the premature newborns and most common forms were GIT (>50%) and cutaneous(35%). The mortality was 85% among the disseminated disease.

Maxson, et al (1992) reported pulmonary blastomycosis in neonates who were developed acute onset of respiratory distress and described the cause was aspiration of vaginal secretion during birth.[83]

Aspergillosis in neonates was reported by **Groll, et al** (1998), that it was uncommon disease in the newborn period and usually manifested as widely disseminated disease. Primary cutaneous disease had been increasing in premature infants.[14]

Malassezia colonization in neonates studied by **Shattuck, et al** (1996) and it was varied from 30-100%. The factors correlated for it were length of hospitalization, duration of central or peripheral venous line, usage of total parentral nutrition(TPN), use of occlusive dressings and lower gestational age. Invasive disease was more among TPN supplemented newborns.[125]

Risk factors :

According to **Roy et al.,** (2002) the most frequent neonatal risk was low birth weight affecting 63.8% of the neonates. Study of maternal risk factors revealed 32.08% of mothers have preterm labor, 28.9% had PROM and 5.2% had intra partum fever. [113]

Shah et al 2006 have reported premature rupture of membranes contributed to 46% of cases of neonatal sepsis in their study. It was associated with early onset sepsis and there was ascending infection from cervico-vaginal epithelial colonization. It was also associated fungal infection from vaginal Candidiasis.[124]

According to **Shabina Ariffet al**,(2011) prolonged mechanical ventilation care more than 7days, , prolonged duration of hospital study more than 7days positive bacterial blood culture report and escalating antibiotic usage without proper antibiotic sensitivity report were the major risk factors for Candidemia in NICU. Antenatal care had a protective impactMechanical ventilation is one of the life saving measures in severe birth asphyxia, preterm neonates with severe RDS, meconium aspiration syndrome with respiratory failure, severe sepsis with multi organ dysfunction, metabolic acidosis and some cases of neonates under post operative care. Such a important intervention may increase the chances of invasive infection and sepsis.[123]

According to **Kristof K et al** (2009), preterm (30weeks), Very Low Birth

Weight (VLBW) babies below 1500gms, Extremely Low Birth Weight (ELBW) babies below 1000gms had increased risk for initial intensive care. These above factors increase the changes of fungal colonization over skin, GIT, and Respiratory mucosa, led to invasive fungal infections and sepsis. Therefore this fungal sepsis would continue to be a challenging complication in neonatal intensive care and also affecting morbidity and mortality.[72]

Signs & symptoms:

According to **WHO Young infant study group**, clinical criteria for diagnosis of sepsis were convulsions, respiratory rate >60 breaths /min , severe chest indrawing, temperature >37.7°C (or feels hot) or <35.5°C (or feels cold), lethargy or unconscious (not aroused by minimal stimulus), reduced movement (change in activity),not able to feed, crepitation, cyanosis and reduced capillary refilling time. Each of the above signs and symptoms were associated with a score, the severity indicated by the increasing level of the score and the prognosis also depending upon the score.[13]

Powell K R (1990) described the earliest signs of sepsis as subtle and nonspecific; poor feeding, diminished responsiveness or just 'not looking well' were the earliest symptoms. More prominent finding are respiratory distress, apnea, lethargy, fever or hypothermia, jaundice, vomiting diarrhea and skin

manifestations including petechiae, abscesses and sclerema.[101]

According to data given by **Gluck et al**(1966), major clinical symptom and signs were hyperthermia 51%, jaundice 35%, respiratory distress 33%, hepatomegaly 33%, lethargy 25%, vomiting 25%, abdominal distention 17%, hypothermia 17%, diarrhea 11%.[41]

Shasikala et al (2000), described the clinical features of sepsis and refusal of feeds (61%), respiratory distress (40%), convulsions(29%), abdominal distention(23%) were the main features in neonatal sepsis.[119]

Diagnosis:

Mary C Harris et al (2007), suggested one septic screen with combination of diagnostic tests which improves the predictive value. That was included absolute neutrophil count <1,750/cmm(1point), Total WBC <7,500 or >40,000/cmm(1point), immmature to total neutrophil count ratio >0.2(1point) or >0.4(2points) and CRP levels >1.0 mg/dl(1point) or >5.0 mg/dl(2points) and the result considered positive when the point value was 2 or more**.**[80]

Yvonne R. Shea,(2011), tabled the specimens for recovery of fungal pathogens. Yeasts like Candida was isolated from all types of samples includes blood, bone marrow, CSF, eye, respiratory sites, skin, mucus membrane, urine, multiple systemic sites and catheter and catheter exit site. Cryptococcus neoformans were isolated from the entire above specimen except catheter and

catheter exit site. Malessazia species were isolated from blood, skin, mucus membrane and catheter and catheter exit site. Moulds like Aspergillus species isolated from brain and CSF, eye, respiratory site, skin, mucus membrane and urine. Zygomycetes were recovered from CSF, eye, respiratory site, skin and mucus membrane.[147]

Kyle C (2008) hand book described about the methods for the diagnosis of candidiasis. Budding yeast cells might be seen in direct wet mount or Gram staining of urine and CSF samples and it should be correlated with that of the culture growth. Blood culture was positive within 3 days after invasive infection. Candida isolation from normal sterile body fluids was considered as invasive fungal infection. Antigen or antibody detection tests were less sensitive in diagnosing fungal infections. Germ tube formation was a simple test used to differentiate C.albicans and C.dubliniensis from other candida species.[73]

Culture:

A study about time to positivity of neonatal blood cultures conducted by **Khadija Guerti et al** (2010) at Belgium, was showed that the isolation of fungal pathogens in suspected sepsis were about 5.5% in a neonatology unit. The time to positivity for yeasts like candida were 40% within 24 hours, 95% within 48 hours and remaining took 72 hours by conventional culture methods.[68]

Arendrup MC, et al (2009), stated that positive blood culture in

invasive fungal infections was only about 40-60% and it was the standard method for diagnosis. In neonates only 0.5-1ml of blood could be withdrawn in each cases and this had reduced the sensitivity of the culture reports. Blood cultures were almost always negative in disseminated Aspergillosis.[6]

Kennaugh et al(1987), documented that common pediatric pathogens of neonatal sepsis might be recovered from small volume of blood (0.5ml),even when it cultured at blood and BHI ratio of 1:100. [67]

Aniscough et al(1998), compared the cost effectiveness of CHROMagar with SDA, and the sensitivity was increased up to 92.5% in CHROMagar. It was more useful in C.albicans isolation on CHROMagar media. It was one of the least time consuming method for presumptive diagnosis. The study suggested direct inoculation of specimens into CHROMagar media when yeast cells were seen in specimens under microscopy.[5]

There were increasing number of patient with invasive fungal infections and more reports were obtained about the resistance pattern to antifungal drugs. To reduce the antifungal drug resistant and for a better clinical outcome, **Sanjay G. Ravenkar** et al (2005) recommended antifungal susceptibility testing for all the isolated fungal pathogen.[117]

According to **Espinel-Ingroff A et al** (1998) many techniques had been used to determine antifungal susceptibility testing like Broth dilution, Agar based

methods, uptake of metabolites and flow cytometry. Agar method was easy and low cost method but there were wide variation of results. Broth dilution methods like macro broth and micro broth were widely used and it had been standardized by CLSI. End point determination was an important observation in broth dilution methods. Amphotericin B the fungicidal drug produce with distinct end point with complete clearance of turbidity but fungistatic drugs like azoles did not produce distinct end point and there was partial clearness of turbidity.[31]

Alternate testing methods described by **Arthington-Skaggs et al** (2002). Simple techniques like disk diffusion and E test and quick methods like flow cytometry, sterol quantization and automated system were used in some laboratory depending upon their feasibility.[7]

Serological test:

Ellepola et al (2005) summarized numerous methods for detection of candida antibodies and antigens. Antibody assays had low sensitivity and low specificity. It could not discriminate systemic candidiasis and colonization. To distinguish the above condition, diagnostic markers of mannan and mannoproteins had been evaluated. Galactomannan was used for the detection of invasive Aspergillosis.[28]

Fungus specific metabolite detection had been used as markers in fungal infection. **Hui M, et al** (2004) described the detection of D-arabinital and D-

mannital and the ratio of D-arabinital /L arabinital by gas liquid chromatography or by mass spectometry.[52]

Philip and Hewitt (1980) documented that measuring the peripheral blood cell count and differential count was probably the most useful non-specific and reliable screening test. If the total count is under 5000 cells/ cmm or if the band to neutrophil ratio equal or exceeded to 0.2, bacterial or fungal sepsis should be strongly considered.[98]

According to **Meharbansingh,** micro-ESR estimation was a simple and inexpensive method but it was not a very reliable marker of neonatal infection. A value of more than 15mm is considered as infection in the neonates. Estimation of C-reactive protein (CRP) level was the best indicator of sepsis and also useful marker to monitoring the effectiveness of treatment.[84]

Biomarkers like acute phase reactants (C reactive protein, feeritin, lactoferrin, neoptrin, procalcitonin and serum amyloidA),cytokines like tumor necrosis factor (TNF) alpha, Interleukins (IL) 1- alpha, 1-beta, 6,8,10,18 and leukocyte surface markers like CD11b,ICAM-1, CD63, CD64, CD66b were used as newer diagnostic tools in some centers and it was stated by Lever A et al (2007).[74]

The CRP level measured at the onset of signs of infection had an overall sensitivity between 35% to 94% and specificity between 60% and 90% in

diagnosis of sepsis as documented by Pourcyrous et al (1993).[100]

Hanan E et al(2012) studied about CD64 expression in neonatal sepsis and noted that it was a useful indicator to differentiate infected from noninfected neonates. This test was done by flow cytometry and the cost of the test was the limiting factor for routine use.[45]

Treatment :

Most of the fungal sepsis were treated by conventional antifungal regimes include amphotericin B and its lipid formulations and fluconazole. In vitro resistance to this drugs, especially for C. albicans and C.parapsilosis were 2to 7% reported by **Pappas et al** (2004). Clinical treatment failure and persistence of infection also reported. Those patients need voriconazole and newer drug echinocandins for clinical cure.[95]

Zaouits et al (2007) suggested amphotericin B treatment for systemic fungal infections. Lipid formulation was useful to reduce renal toxicity. Azoles like fluconazole and voriconazole were also used as alternate for amphotericin B. Combined use of flucytosine with amphotericin B had better tissue penetration. Flucytocine monotherapy might lead to resistance. After negative culture report for fungal growth, the antifungal treatment to should be continued for further 14 days.[150]

Juster-Reicher et al (2003) recommended intravenous infusion of amB deoxycholate at a dose of 1.0 to 1,5 mg / kg / day over 2-6 hours and liposomal formulation at a dose of 5 to 7 mg / kg / day over 2 hours. Addition of oral flucytosine 12.5 to 37.5 mg /kg / dose for every 6 hours was helpful if there was meningitis. Empiric antifungal was also recommended for acutely thrombocytopenic ELBW infants**.**[60]

Yang YI et al, (2003) studied about antifungal drug resistance and noted the susceptibility degrees of candida species towards the antifungal drugs varied and there was increased resistance to antifungal drugs.[146]

Sturat SM et al (1992) suggested temporary cessation of lipid infusions and removal of central venous catheter (CVP) to control the growth of malassezia infection. Ampotericin B should be used for treatment until the negative blood culture report.[132]

Espineal-Ingroff et al (2008), stated that, in vitro antifungal susceptibility test were provide a reliable measure of the relative activities of antifungal drugs, correlate in vivo activity and predict the outcome, to monitor the development of drug resistance and predict the therapeutic potential of newer drugs. New developments in the standardization of in vitro antifungal susceptibility testing procedures become a useful aid in selecting appropriate drugs. Apart from drug in vitro susceptibility, host immune response, status of underlying disease, proper

diagnosis and management and interaction of organisms and drug inside the patient are also determine the outcome.[30]

Sandana et al., (1997), have evaluated the role of double volume exchange transfusion in septic neonates with sclerema and demonstrated a 50% reduction in sepsis related mortality in the treated group. That also found, non-specific pooled intravenous Immuno globulin not a beneficial one.[115]

Prevention:

Mathus et al., (1990) documented that, regular antenatal check up during pregnancy and increased use of prenatal care facilities might lower the rate of prematurity. More appropriate management of prolonged interval after rupture of membranes, maternal post partum infections and fetal distress would reduce the incidence of neonatal sepsis.[81]

Harbarth S et al (2002), suggested hand washing and degerming by alcohol based hand rub with emollient were the simplest and most effective methods of preventing transmission of infectious agents from health care personal to newborns based on their study in hospitals and NICUs.[49]

The **NeoIN** Surveillance network in England (2011) implemented simple infection control methods with proven efficacy. The suggested methods by their surveillance study were s promotion of clean deliveries, hand washing and barrier nursing, exclusive breast feeding, restriction of antibiotic use and minimizing

intervention and rationalization of admissions and discharges from neonatal units.[137]

Adams-Chapman et al,(2002) suggested important principles for the prevention of nosocomial infection in NICU and that were nursery design engineering, Universal precautions, hand washing, meticulous skin care, minimizing central venous catheter contamination, early and appropriate advancement of enteral feeding , education and feedback for nursery personnel and continuous monitoring and surveillance of nosocomial infection rate in NICU.[1]

MATERIALS AND METHODS

Materials and Methods

This study was conducted at Government Rajaji Hospital attached to the Madurai Medical College, Madurai. The study period was one year from September 2013 to September 2014. Permission was obtained from Director, Institute of Child Health and Research Centre (ICH & RC), and Professor, Department of Paediatric Surgery ,GRH. A total of 200 suspected cases of neonatal septicaemia were included in this study. Ethical committee clearance was obtained before starting the study and informed consent was obtained from parent or guardian of each patient.

The samples like blood, urine and cerebrospinal fluid (CSF) were collected from neonates admitted at Newborn Intensive Care Unit (NICU).

Inclusion criteria

1. Newborn babies more than 1 week of NICU stay.
2. Babies admitted with suspected septicemia.
3. Premature babies (37 weeks), low birth weight babies (<2500gms).
4. Babies on ventilator care.
5. H/O insertion umbilical line /Presence of central venous catheter
6. Use of surfactant / blood, blood products or exchange transfusion / on total

parentral nutrition (TPN).

7. Newborn with broad-spectrum antibiotics administration.

8. Neonates who underwent major surgeries.

Exclusion Criteria :

1. Newborns with short duration of NICU stay (<7days)

2. Newborns admitted for observation

3. Admitted for phototherapy

4. Newborns with major congenital anomalies.

5. Other healthy babies

Selection of cases:

Newborns with above mentioned criteria were included in this study. Data like date and time of delivery, mode of delivery (normal, LSCS, outlet forceps and vacuum extraction), duration of labour, place of delivery (home, primary health centre (PHC), Government hospital (GH), private hospital and institutional delivery), transport details if any and admission time with date were recorded.

Infants risk factors like low birth weight, preterm, surfactant therapy, ventilator care, total parental nutrition (TPN), duration of hospital stay and broad spectrum antibiotic administration, history about umbilical catheter insertion, central line intravenous catheter, history about major surgical intervention, transfusion of blood, platelet, fresh frozen plasma (FFP) and exchange transfusion

were noted..

Investigation reports like total count, differential count, immature cell count, total vs. immature cell ratio (I/T), platelet count, C-reactive protein level (CRP) and previous bacterial culture report if any were noted from the case records of the newborns

Sample Collection:[86]

Sample like blood, CSF or urine were collected from the clinically selected cases for fungal culture.

Blood:

Blood sample was collected under strict aseptic precautions. Approximately 5 cm diameter of the skin over the selected site was cleaned thoroughly with 70% ethyl alcohol or isopropyl alcohol, followed by 10% Povidone iodine and then the skin was allowed to dry for at least one minute before the sample was collected. About 0.5- 1ml of blood was collected and it was inoculated into the Brain Heart Infusion (BHI) broth bottles (5 ml BHI in each bottles) for conventional culture. All the blood samples were collected from a peripheral vein and not from pre-existing intravenous catheters.

CSF:

Adapting strict aseptic precautions, CSF samples collected by lumbar puncture method (L3-L4 level of spinal card) in suspected babies. 1-2 ml of CSF

collected in a sterile screw capped tube. It was transported immediately to the laboratory for wet mount, Gram staining, India ink mount and for culture.

Urine:

Urine sample was collected aseptically by insertion of sterile urinary catheter or infant feeding tube and it removed immediately after the adequate volume of urine (10 -20 ml) collection in suspected septicemia cases of newborns. Urine was collected in a sterile container and transported immediately to laboratory for processing.

Transport and storage:

All the bottles or containers were checked for proper labeling and requisition forms were filled with necessary details of each patient.

All the samples were collected in a leak proof container and transported to the laboratory as early as possible. If there was delay in transport of sample, blood and CSF were kept in room temperature and urine was refrigerated at 4°C.[86]

Processing of samples:[56]

The specimens were processed as per protocol, depending upon nature of material collected in a particular case. Universal precautions were followed during the processing.

The entire samples were examined macroscopically for the turbidity,

presence of fungal filaments and deposits. Sabourauds Dextrose Agar (SDA) medium with antibiotic supplements- gentamicin and cholramphenical was used for fungal culture.

Blood:

The blood samples in BHI broth was directly inoculated in to two SDA slopes .

CSF:

If the volume was below 2ml it was directly used for processing. If it was above 2ml, it was concentrated by centrifugation (1500-2000 rpm for 3 minutes), the supernatant was removed aseptically with a sterile pipette and the sediment was used for processing like direct wet mount, Gram staining, India Ink examination and directly inoculated in to two SDA slopes for culture.

Urine:

Urine sample was centrifuged at 2000rpm for 3 minutes. The supernatant was removed aseptically with a sterile pipette and the sediment was used for processing. It was used for direct wet mount, Gram staining and inoculated in to two SDA slopes for culture.

Direct microscopic examination:[53]

Direct wet mount:

1. The specimens like centrifuged CSF and Urine deposits were examined as

direct wet mount.

2. A drop of the specimen (centrifuged deposit of CSF and urine) was placed on a clean glass slide.

3.It was covered with a cover slip and observed under microscope at low and high power objectives.

Gram staining:

Gram staining is effective for detection of some of fungal pathogens directly from specimen. The yeast cells are Gram-positive in stained smear.

Procedure:

1. Smear was prepared from centrifuged CSF and urine sample.

2. After fixing the smear, 0.5% aqueous crystal violet solution poured over the slide to cover the smear and kept for 1 minute.

3. It was washed gently under tap water.

4. Gram's iodine solution was applied over slide for 1 minute and washed with tap water.

5. It was decolorized quickly with acetone (2 to 3 sec) and washed immediately in water.

6. Counter staining was done with dilute carbol fucshin and washed with tap water.

7. Air or blot dry was done and examined under the oil immersion.

All fungi are Gram positive. The yeast cells usually show well stained morphology but filamentous fungi in smears become desiccated and their morphological characteristics may be lost.

India ink preparation:

Permanent black fountain pen Ink was used for this test. Procedure:

1. A drop of the specimen (centrifuged deposit of CSF, urine) was placed on a clean glass slide.

2. 1-2 drops of India ink was added and mixed. The preparation should be brownish, not black.

3. It was covered with a cover slip and observed under low and high power objectives.

Capsular material is exhibited by the appearance of a clear, well demarcated halo around the yeast cell.

The capsules of cryptococci vary from 2 to 10µm or more in width.

White cells was distinguished from Cryptococcus neoformans because of the irregular edge with halo and a pale cell wall in WBC. [53]

Lacto Phenol Cotton Blue (LPCB) mount:

LPCB mount is effective for detection of fungal morphology especially in moulds.

Procedure:

1. A drop of LPCB was placed on a clean glass slide.

2. A drop of the specimen (centrifuged deposit of CSF, urine) was mixed with LPCB.

3. It was covered with a cover slip and observed under the microscope with low and high power objectives.[53]

Fungal culture: [55]

Emmons' modification of Sabouraud Dextrose Agar (SDA) was used for culture. The media supplemented with antibiotics, such as gentamicin and chloramphenical.

1. SDA slants were prepared in a sterile screw capped bottles.

2. Each sample was inoculated in to two SDA slopes and they were incubated at two different temperature , one at 37°C and another at 25°C.

3. The growth was observed every day for first three days, 5thday and 7th day of first week and then every week for four weeks duration.

4. When fungal growth was noted, that SDA growth was subjected for subculture and further processing. It was observed for entire period of four weeks to six weeks.

Culture reading: [56]

Macroscopic appearance of growth:

Yeast colonies were smooth, creamy, viscous or pasty colonies and grown within 24-72 hours.

Moulds were distinct woolly or cottony colonies and aerial mycelium were also noted in some of the fungal growth.

Rapidly growing moulds (48-72 hours) without an distinct border, Zygomycetes may be considered.

Moulds that grow within 3-5 days, had a distinct border and white, pastel on surface with new growth at the periphery were belongs to hyaline group of moulds.

Slow growing (7-10 days) with a delicate or cobweb aerial mycelium usually gray or gray-brown in color considered as dimorphic fungi.

Identification methods:

These include microscopic examination, special culture techniques, and specific tests for speciation, biochemical reactions and antifungal susceptibility testing of the isolates.

Microscopic examination:

Direct wet mount, India ink preparation and Gram staining was done from the fungal growth as described above.

Lacto Phenol Cotton Blue (LPCB) mounts:

LPCB mount effective for detection of fungal morphology especially in moulds.

A small portion of the colony removed from the agar surface with a teasing needle and placed it into the drop of LPCB. The mycelial mass was teased and separated apart by using pairs of teasing needles. It was covered with cover slip and examined as described above.

Colony morphology:[54]

Candida yeast cells are small oval cell with single budding and Gram positive. On SDA cream colored pasty colonies seen after 24-48 hours at 35-37 ℃. It had a distinctive yeast smell.

Candida growth morphology:

S.No	Species	Growth on SDA
1	C.albicans	Cream colored, pasty and smooth, edges are radiating starburst spicules in old culture.
2	C.tropicalis	Cream to off-white, glistening to dull, soft, smooth or wrinkled with mycelial fringe.
3	C. glabrata	Small, glistening, smooth, cream-colored.
4	C. krusei	Flat, dull and dry in long incubation greenish yellow, wrinkled.
5	C.kefyr (pseudotropicalis)	Creamy, smooth to yellowish, dull.

6	C. parapsilosis	Creamy, lacy pattern.
7	C. guilliermondii	Creamy, smooth to yellowish, dull.
8	C. parapsilosis	Creamy, lacy pattern

Speciation of candida:[69]

For identification and speciation of Candida, Germ Tube Test (GTT), Dalmau plate method on Corn Meal Agar (CMA), urease test, sugar fermentation and assimilation tests (auxanogram), were used. GTT was used for presumptive identification of C.albicans and C. dubliniensis. The demonstration of germ tube is also known as **Reynolds-Braude** Phenomenon. The above two also produce chlamydospores on CMA or Rice Meal Agar (RMA). C.dublinensis does not grow at 45 ℃ and does not assimilate xylose sugar. C.glabrata ferments and assimilates only glucose and trehalose, whereas C.tropicalis do so with sucrose and maltose.

Morphological features of yeast on CMA often allow tentative identification of particular species. CHROMEagar Candida is a rapid plate based method used for simultaneous isolation and identification of various Candida species. This is based on the direct detection of specific enzymatic activities by adding certain fluorochrome substrate to the media.

Tests used for identification of yeasts

1. GermTubeTest[56]

A germ tube is defined as a filamentous extension from a yeast cell that is about half the width and three to four times length of mother cell. It is a hyphal structure and there is no constriction at the neck.

1. In a sterile test tube, 0.5 ml of human serum was taken.

2. With a sterile loop, small portion of yeast colony was inoculated into the serum.

3. It was incubated at 37 °C. for 2-3hours.

4. After incubation, wet mount was prepared from that suspension.

5. It was observed under low and high power objectives of a microscope.

Candida albicans and C.dubliniensis produce germ tube.

Germ tubes are the beginnings of true hyphae and appear as filaments that are NOT constricted at their points of origin from the parent cell. If the filaments are constricted and septate at their points of origin they were considered as pseudohyphae, not germ tubes.

2. **DALMAU PLATE(**Corn meal agar) morphology: [35]

Corn Meal Agar with tween 80 was used to differentiate species of Candida.

With a sterile loop, small portion of yeast colony was inoculated vertically on to CMA as a streak line without cutting the agar. Then 3 to 4 streak lines were made across the first inoculation to dilute the first inoculam. The streak lines were covered with a 22 by 22mm cover slip. It was incubated at room temperature in the

dark for 24-48 hours. The growth was examined microscopically after removing the lid and placing the culture plate directly under the low and high power objectives. An isolate of C.albicans was included as a positive control.

DALMAU PLATE (Corn meal agar) morphology:

S.No	Species	Morphology on Cut-streak (Corn Meal) agar (DALMAU PLATE)
1	C.albicans	Irregular or spherical clusters of blastospores at septa. Numerous chlamydospores single or in clusters, Terminal chlamydospores.
2	C.tropicalis	Blastospores anywhere along mycelium or in irregular clusters. Chlamydospores very rare
3	C. glabrata	Very elongate cells which readily fall apart and lie parallel. "Logs in stream" appearance, not produce pseudohypha
4	C. krusei	Elongate cells forming a branched mycelium easily disintegrated. "Crossed sticks" of septa.
5	C.kefyr (pseudotropicalis)	Very elongated cells, abundance of pseudohyphae
6	C. parapsilosis	Fine and coarse mycelium (giant forms). Blastospores single or in short chains at septa or distal ends of cells

7	C. guilliermondii	Very fine mycelium. Small clusters of blastospores at the septa
8	C.dubliniensis	Irregular or spherical clusters of blastospores at septa. Numerous chlamydospores single or in clusters,

3.Sugar fermentation tests: [147]

It is a method used for identification of yeasts by the ability to ferment various sugars.

1. Fermentation test media was prepared by using sugars like like glucose, sucrose, lactose and maltose, galactose and trehalose in a sterile test tube with inverted Durham's tube.

2. Heavy inoculam of yeast colony grown on sugar free media was used for preparation of suspension.

3. Each carbohydrate broth was inoculated with approximately 0.1 ml of above suspension.

4. All test tubes were incubated at room temperature up to one week.

5. The tubes were examined daily for production of acid (yellow color) and gas in Durham's tube.

6. Production of acid and gas was taken as fermentation reaction and only acid production indicates the assimilation.

Interpretation of fermentation tests:

S.No	Species	Glu	Mal	Suc	Lac	Gal	Tre
1	C.albicans	AG	AG	A	-	AG	AG
2	C.tropicalis	AG	AG	AG	-	AG	AG
3	C. glabrata	AG	-	-	-	-	AG
4	C. krusei	AG	-	-	-	-	-
5	C.kefyr (pseudotropicalis)	AG	-	AG	AG	AG	-
6	C. parapsilosis	AG	-	-	-	-	-
7	C. guilliermondii	AG	-	AG	-	AG	AG
8	C.dudliniensis	AG	AG	-	=	AG	AG

Note: Glu = Glucose, Mal = Maltose, Suc = Sucrose, Lac =Lactose, Gal = Galactose, Tre = Trehalose, A = Acid Production, G = Gas Production.

4. Sugar assimilation tests:[148]

(Auxanographic plate method - Haley & Standard modification):

1. Yeast suspension was prepared by mixing of yeast colonies in 6 ml of distilled water. The turbidity was matched with No 4 Mcfarland standard

2. Yeast nitrogen base medium was prepared and allowed to decrease the temperature to 50°C.

3. Two sterile disposable petri dishes were labeled with number of each

isolate and the names of sugars used (6 sugars per plates).

4. Three ml of yeast suspension and 15ml of yeast nitrogen agar base poured to the above petri dishes and mixed well.

5. It was allowed to settle at room temperature.

6. Carbohydrate solution (20%) was prepared separately for each sugar.

7. Sterile discs were made from Whatman No1 filter paper. 6 discs were placed over the agar medium of each plate.

8. By using a 2 mm sterile loop, 20% carbohydrate solution was placed over the discs in the designated areas.

9. The plates were incubated for 24 hours at room temperature.

10. The plates were observed for growth of yeast colonies around the discs. If there was growth around particular sugar disc, then that sugar was taken as assimilated.

The sugars used for assimilation test were: Glucose, Maltose, Sucrose, Lactose, Cellobiose, Galactose, Trehalose, Raffinose, Melibiose, Xylose, Inositol and Dulcitol.

5. Kit based Sugar assimilation tests:

This test was done by using a standardized colorimetric identification kit with 12 conventional biochemical tests. Eleven tests for sugar assimilation and one for urea hydrolysis. HiCandida Identification Kit obtained from HiMedia

laboratories Pvt. Limited, Mumbai. The tests were based on the principle of pH change and substrate utilization. Growth of a particular species produced metabolic changes which were indicated by a spontaneous color change in the media.

Procedure:

1. A single fresh yeast colonies (24-48 hours) from SDA was inoculated into 5 ml of Potato Dextrose Broth (PDB).

2. It was incubated at 22-25 °C for 6-8 hours and turbidity was matched to 0.5 McFarland standards (1 X 10□ to 5 X 10□ CFU/ml).

3. Under aseptic condition, the test strip was opened. Lab ID Number was marked at a corner.

4. There were 12 test wells in one kit. Each well of the strip was inoculated with a loopeful of inoculum from PDB tubes by stabbing.

5. Then it was incubated at 22-25°C for 24 to 48 hours.

6. Test results were interpreted after 24-48 hours, yellow indicated positive reaction and orange indicated negative reaction, pink color change in urease test well indicated hydrolysis of urea.

Sugar assimilation test done by conventional and kit based method were compared, the results were same.

Interpretation of assimilation tests:

S. No	Species	Glu	Mal	Suc	Lac	Gal	Mel	Cel	Ino	Xyl	Raf	Tre	Dul
1	C.albicans	+	+	+	-	+	-	-	-	+	-	+	-
2	C.tropicalis	+	+	+	-	+	-	+	-	+	-	+	-
3	C.glabrata	+	+	-	-	-	-	-	-	-	-	+	-
4	C.krusei	+	-	-	-	-	-	-	-	-	-	-	-
5	C.parapsilosis	+	+	+	-	+	-	-	-	+	-	+	-
6	C.guillermondi	+	+	+	-	+	+	+	-	+	+	+	+
7	C.kefyr	+	-	+	+	+	-	+	-	+	+	-	-
8	C.dubliniensis	+	+	+	-	+	-	-	-	+	-	+	-

Note: Glu = Glucose, Mal = Maltose, Suc = Sucrose, Lac =Lactose, Cel = Cellobiose, Gal = Galactose, Tre = Trehalose, Raf = Raffinose, Mel = Melibiose, Xyl = Xylose, Ino = Inositol, Dul = Dulcitol; + = Positive Reaction, - = Negative Reaction, V = Variation.

6.Urea hydrolysis [56]

This test was used to detect the urease enzyme production ability of yeasts. The yeast colony was inoculated on slants of Christensen's urea agar and incubated at 25-30°C. Urease enzyme splits urea into ammonia which raises the PH of the

medium and changes the color of the phenol red indicator to pink color. Positive test was indicated by change in color to deep pink within 2-5 days. All of the Candida species give negative results except C.krusei.

7.CHROMagar Candida[29]

CHROMagar test media was a selective and differential chromogenic medium. The test was based on direct detection of specific enzymatic activities of different Candida species. This CHROMagar test media contains substrate of flourochrome dyes which produce different colors for different species of Candida growth to the media. Media was brought from HiMedia laboratories Pvt. Limited, Mumbai (HiCHROM Candida Differential Agar).

Procedure:

Yeast suspension was prepared from fresh colonies of Candida growth. It was inoculated to CHROMagar media by streak culture method and incubated at room temperature .The plates were observed everyday up to one week for color production.

Interpretation of CHROMagar Candida medium growth color:

C.albicans: Light green to Bluish green

C.tropicalis: Blue with pink halo

C.glabrata: Pink to purple, glorry

C.krusei: Spreading pink

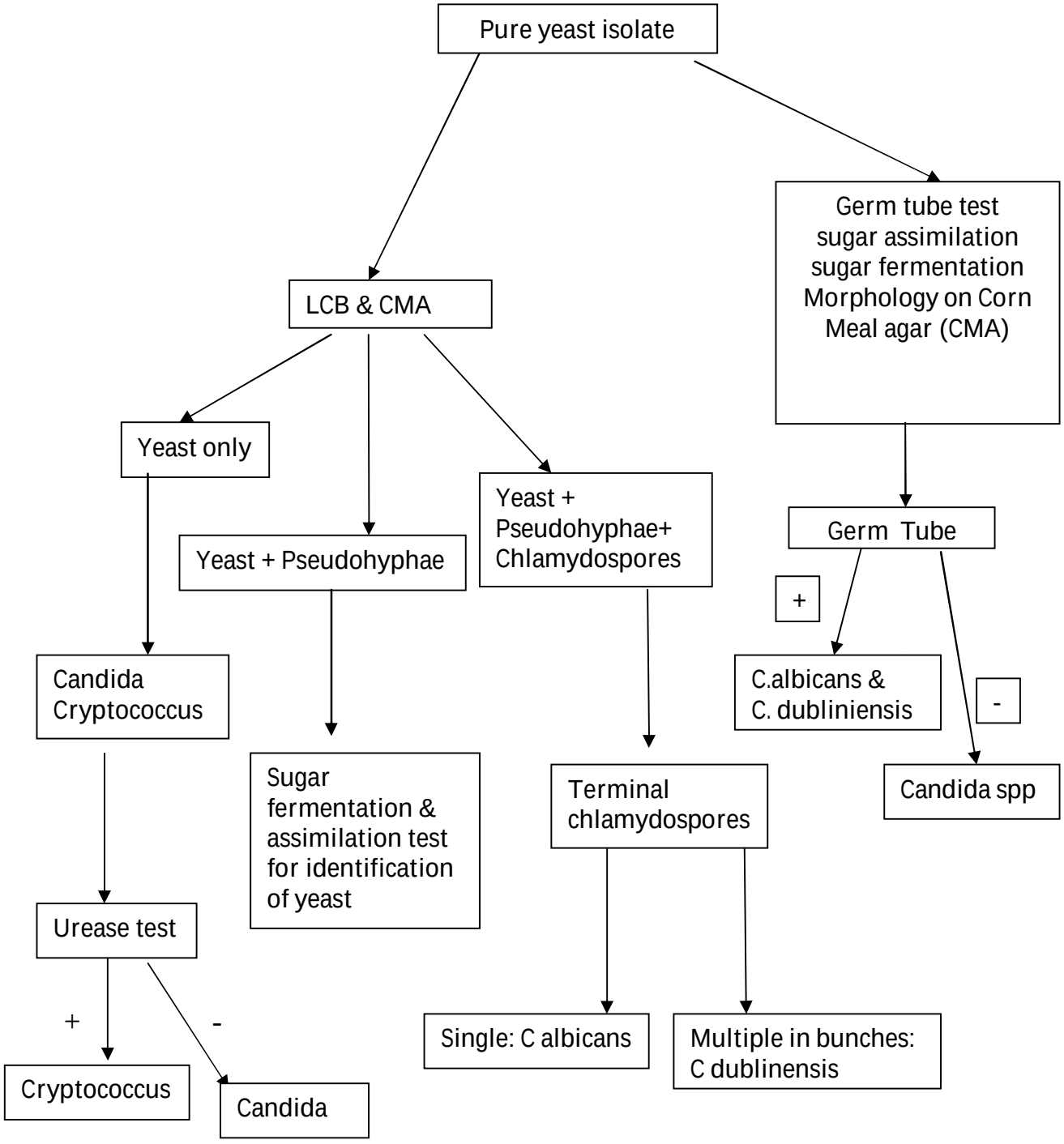
C.kefer : White

C.parapsilosis : Cream/off-white

C.guilliermondii: White to cream

C.dubliniensis: Dark green

Yeast Identification Scheme:



ANTIFUNGAL SUSEPTIBILITY TESTING METHODS

Methods used for antifungal susceptibility testing

S.No	Test method	Endpoint detection
1	Broth macrodilution	Visual comparison of turbidity (≥50% inhibition)
2	Broth microdilution	Visual comparison of turbidity (≥50% inhibition)
3	Agar dilution	Visual comparison of growth
4	Disk Diffusion	Zone diameter.

1.Macro broth and Micro broth Dilution antifungal susceptibility Testing[106]

Purpose

Antifungal susceptibility testing is conducted to determine the minimum concentration of antifungal drug required to inhibit growth of fungal isolates. The macro broth dilution method was the first standardized method but it was very labor intensive. This led to the development of the Microtitre method. Microtitre wells are inoculated with a standardized inoculum from nutrient broth suspension (RPMI1640 for fungi) in columns 1-11 while columns 12 acts as the media negative control. The primary advantage of such broth dilution tests is that it permits several organisms to be tested per plate.

Clinical significance:

Antifungal susceptibility testing assists the physician in making therapeutic decisions. It is also useful in identifying potential reasons for therapeutic failure.

All yeast isolates must be streaked for isolation onto Potato Dextrose agar (PDA). Subculture yeast to PDA agar quadrants and incubate at 30°c for 24-72 hours prior to testing.

CLSI M27-A3 document broth dilution guidelines for antifungal susceptibility testing of yeasts.

Procedure:

1.Broth medium was RPMI 1640 broth buffered with MOPS buffer (0.165 M) and 0.2% dextrose and glutamine without sodium bicarbonate to a pH of 7.0. Media was brought from HiMedia laboratories Pvt. Limited, Mumbai

2. Inoculum was prepared from the fresh growth on Potato Dextrose agar (PDA). Five colonies were picked from PDA and mixed well into RPMI medium.

3. Stock inoculum suspension adjusted to match the turbidity of a 0.5 McFarland standard (1 ✗ 10□ to 5 ✗ 10□ CFU/ml) .

Procedure	Macro dilution	Micro dilution
Test inoculam preparation	A dilution of 1:2000made from stock inoculum suspension; inoculum size after inoculum, 0.5✗ 10^3 to 2.5✗ 10^3 CFU/ml.	A dilution of 1:1000made from stock inoculum suspension; inoculum size after inoculum, 0.5✗ 10^3 to 2.5✗ 10^3 CFU/ml.
Drug dilution	Done by adding drug with additive (DMSO) 10 ✗ dilutions for Fluconazole and drug with solvent (DMSO) 100 ✗ dilution for Amphotericin B.	Done by adding drug with additive (DMSO) 2 ✗ dilutions for Fluconazole and drug with solvent (DMSO) 100 ✗ dilution for Amphotericin B.
Drug dilution range for testing	Drug dilution ranges were for fluconazole 0.12-64 µg/ml and for amphotericin B 0.03-16 µg/ml	Drug dilution ranges were for fluconazole 0.12-64 µg/ml and for amphotericin B 0.03-16 µg/ml
Drug and inoculam mixing	0.1 ml of drug and 0.9 ml of inoculam added to small sterile test tubes. 10 tubes for different drug	100µl of drug and100µl of inoculum added to microtitre well. 10 wells for different

	range ,11th tube for inoculam control and 12th one for media control .	drug range ,11th well for inoculam control and 12th one for media control.

Reading and interpretation:

All tubes or microtitter wells were incubated at room temperature.

Time of reading for Amphotericin B, 24 or 48 hours and for fluconazole 48 hours.

MIC by visual examination measured for Amphotericin B as lowest drug concentration that prevents complete growth (100% inhibition). MIC by visual examination measured for Fluconazole as lowest drug concentration that shows prominent (~50%) decrease in turbidity.

Agar dilution method: [22]

Medium:

Agar dilution method was performed on SDA plates supplemented with gentamicin and chloramphenical.

Procedure and interpretation:

1. Drug dilution done by adding drug with additive (DMSO) 10 X dilutions for Fluconazole and drug with solvent (DMSO) 100 X dilution for Amphotericin B.

2. From each range of drug dilution 2 ml was taken and added to 18 ml of molten SDA and then poured into petri dishes and allowed to settle.

3. The SDA plates were divided into 9 columns by using marker pen and 8 columns were used for test organism and 9th one for sterility control.

4. 10µl of standardized inoculum was added to the corresponding column. About 8 isolates were taken for each test and that inoculated to 8 columns and 9th column was kept uninoculated.

5. Plates were incubated at room temperature for 24 hours.

6. All the plates were inspected macroscopically for the presence of growth.

7. Lowest concentration of the drug which prevented growth was taken for MIC.

Interpreting Results:-

Antifungal agent	Concentration tested	Organism	Reportable reading conditions	Break points		
				Susceptible	S-DD	Resistant
Amphotericin B	0.03 - 16 µg/ml	All Candida species	100% inhibition of growth after 24hrs	≤1.0 µg/ml	-	>1.0 µg/ml
Fluconazole	0.125 - 64 µg/ml	All Candida species except	50% inhibition of growth after 24hrs	≤2 µg/ml	4 µg/ml	≥8 µg/ml

		C.glabrata				
		C.glabrata	24hrs 50%	-	≤3 2 µg/ ml	≥64 µg/ml

Note: C.kursei - intrinsic resistance to fluconazole.

S-DD - Susceptible Dose Dependent.

Disk diffusion susceptibility testing :[21]

CLSI M44-A2 document guidelines for antifungal disk diffusion susceptibility testing of Candida species.

Procedure and interpretation:

1. Agar medium used was Mueller-Hinton agar + 2% dextrose and 0.5 µg of methylene blue dye/ml

2. Inoculum preparation done by picking up five colonies from 24-h growth (Candida spp.) on potato dextrose agar

3. Test inoculum was prepared from stock inoculum suspension, adjusted by spectrophotometer at 530 nm to match the turbidity of a 0.5 McFarland standard which was equal to 1×10^6 to 5×10^6CFU/ml

4.Test inoculmn was inoculated on to agar medium by pour plate method.

5.Disks used with contents of Fluconazole 25 mcg; Itraconazole 10mcg;

Voriconazole 1mcg, Amphoteracin B 20 mcg and Nystatin 50mcg.

5.All the 5 discs were placed on to agar medium and incubated for 20-24 h at room temperature.

6. Zone diameter was measured as the nearest point at which there was prominent reduction in growth. Pinpoint micro colonies at the zone edge or colonies within the zone should be considered as sensitive.

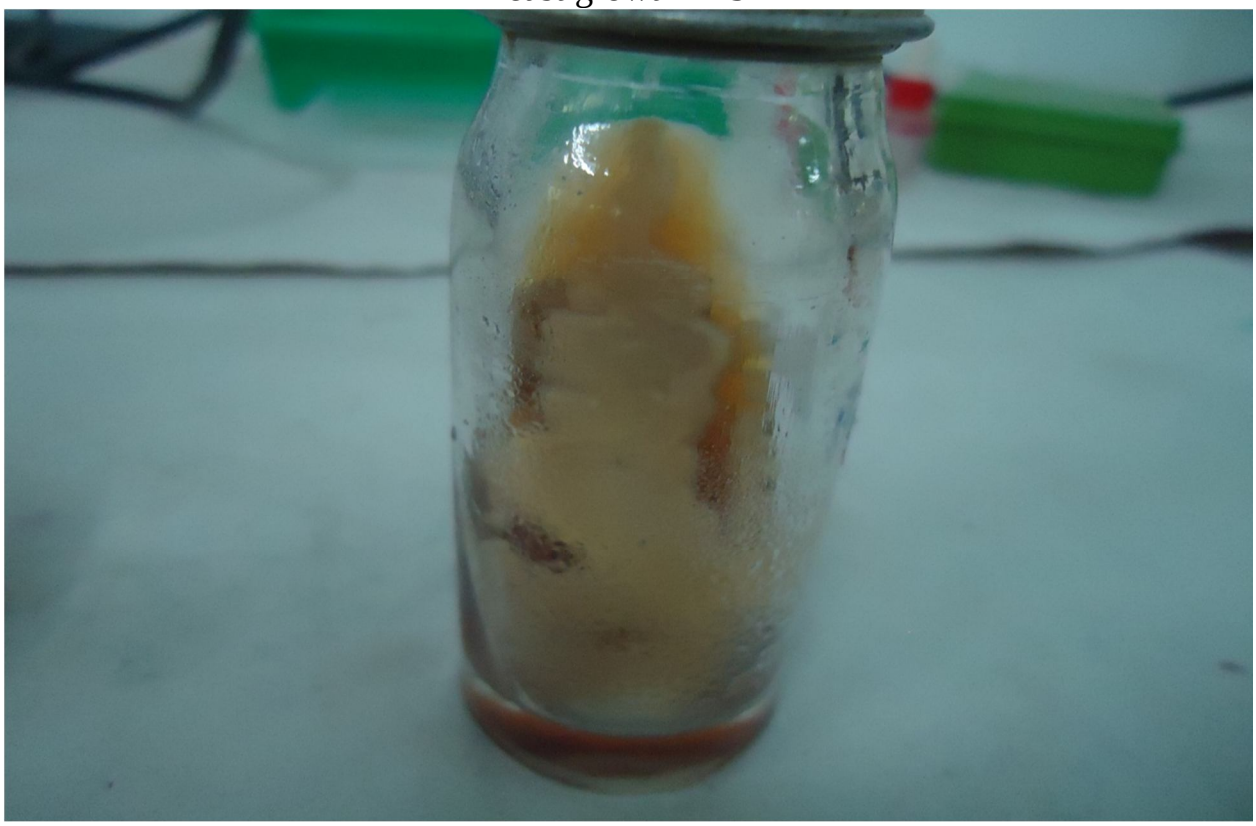
Zone size interpretative chart

(Mueller-Hinton agar + 2% dextrose and 0.5 µg of methylene blue dye/ml)

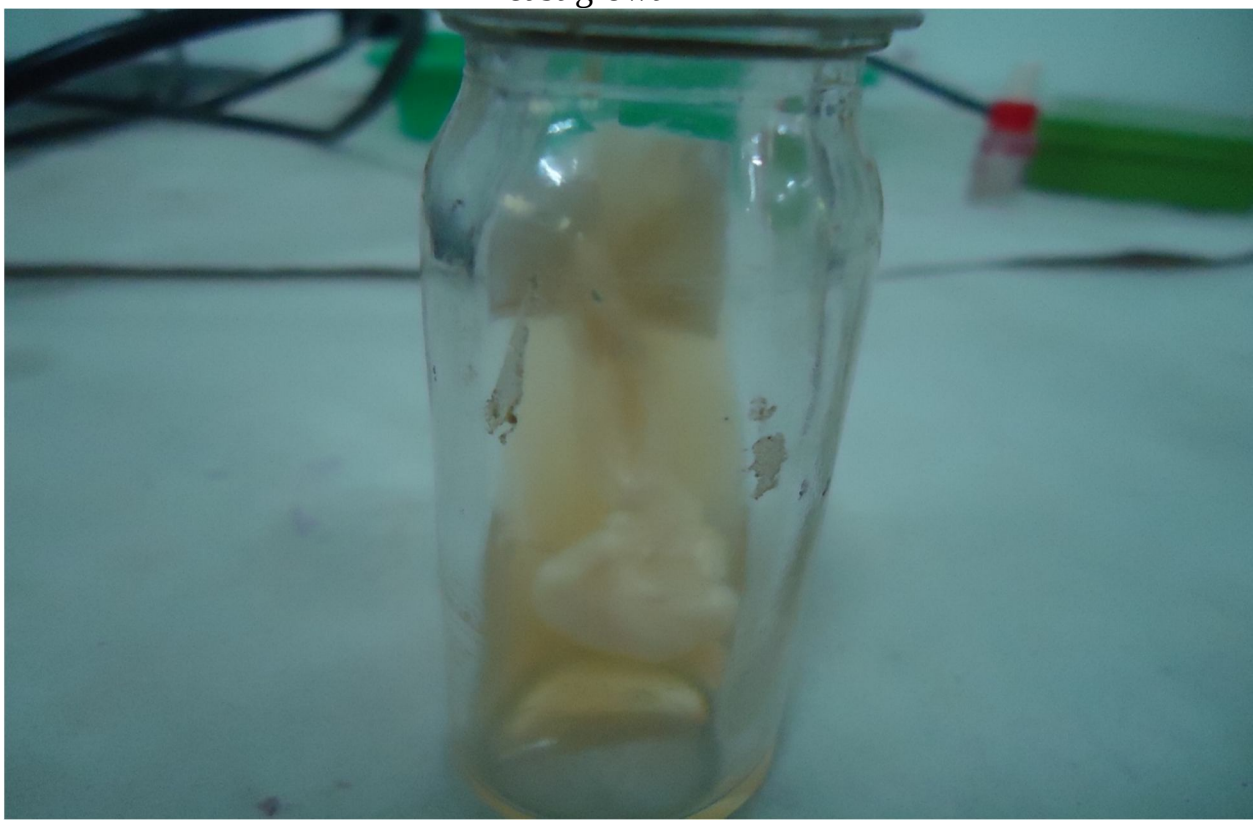
Antifungal agent	Disc content	Zone diameter, Nearest Whole (mm)		
		Resistant mm or less	S-DD	Sensitive mm or more
Amphotericin B	20mcg	9	10-17	17
Fluconazole	25mcg	14	15-18	19
Itraconazole	10mcg	15	16-20	21
Voriconazole	1mcg	13	14-16	17
Nystatin	50mcg	18	19-23	23

Note: S-DD -Susceptible Dose Dependent.

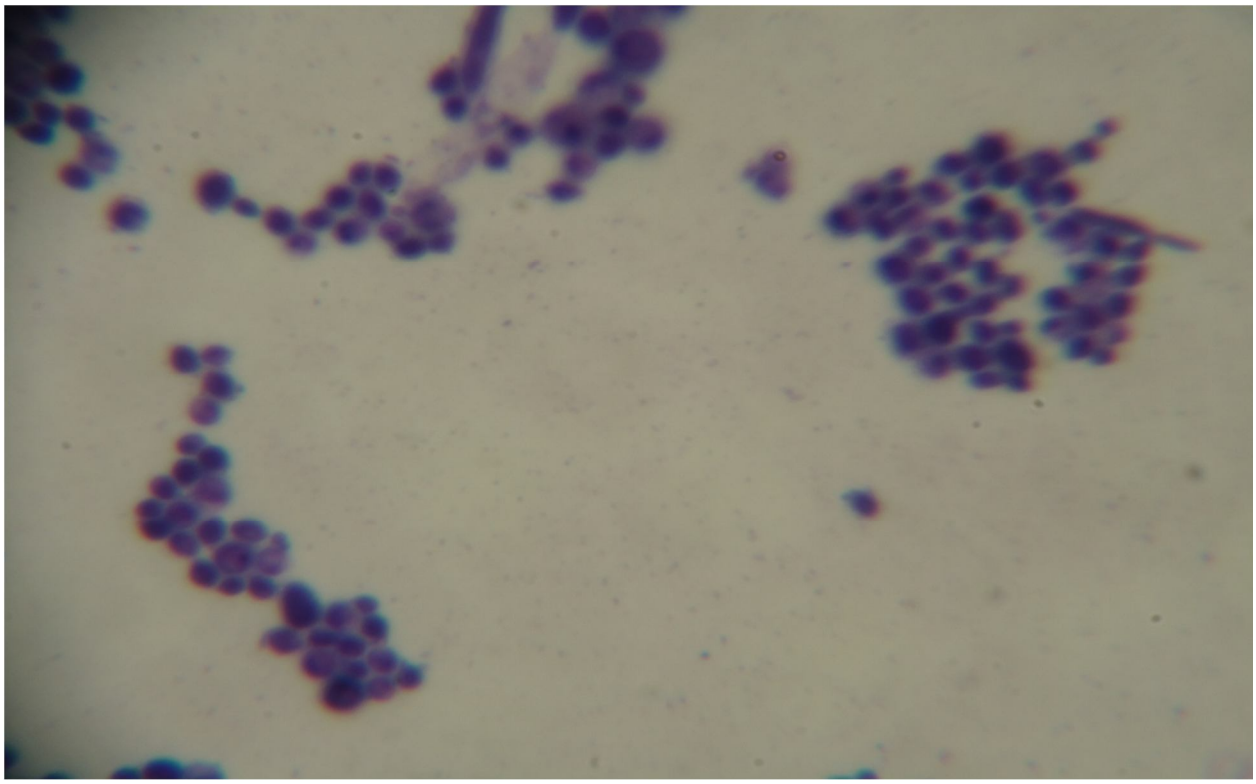
Yeast growth in SDA



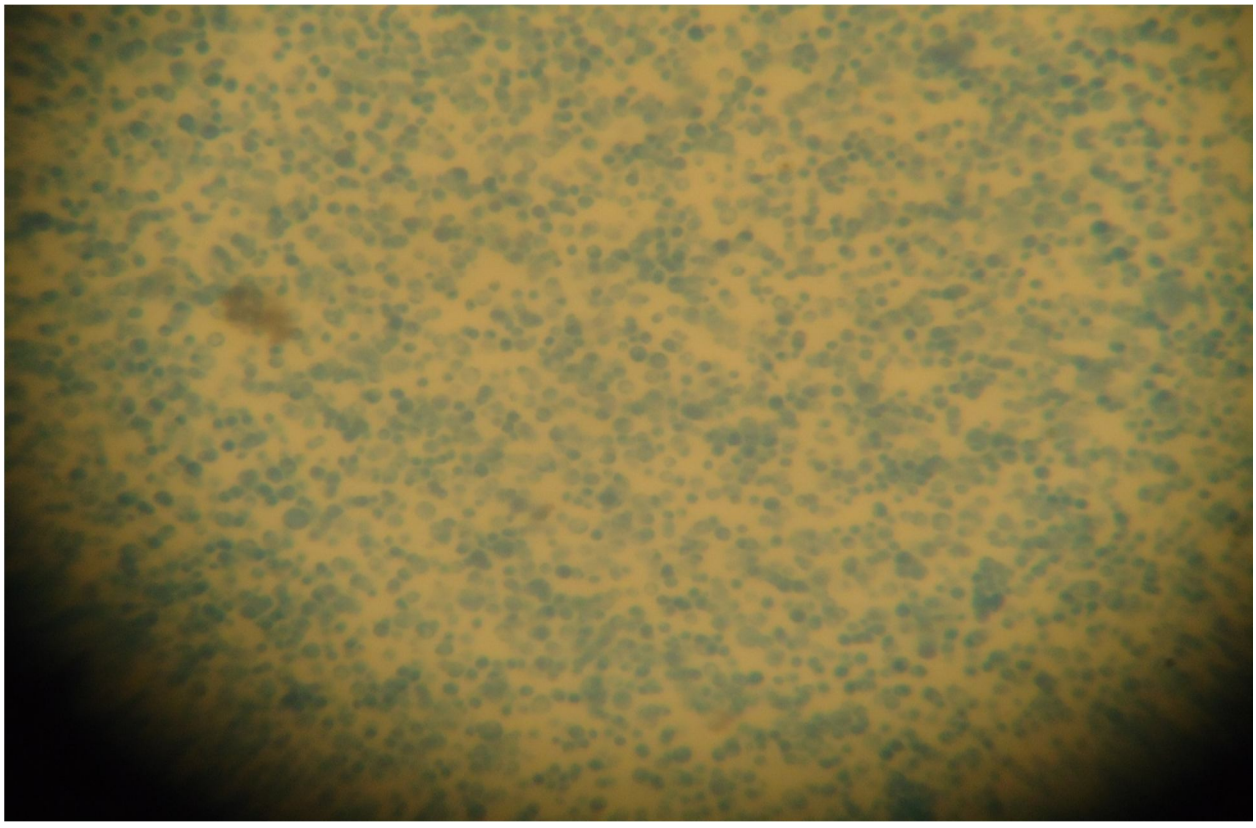
Yeast growth in PDA



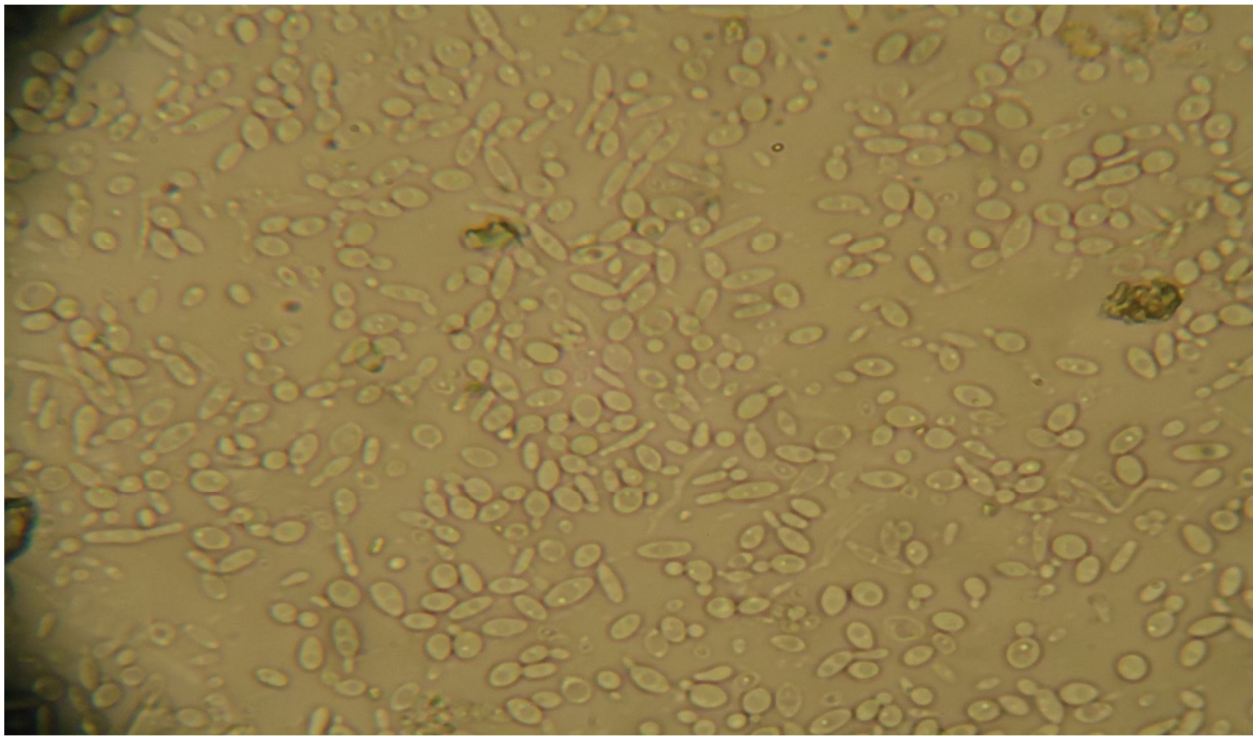
Yeast cells in Gram stain



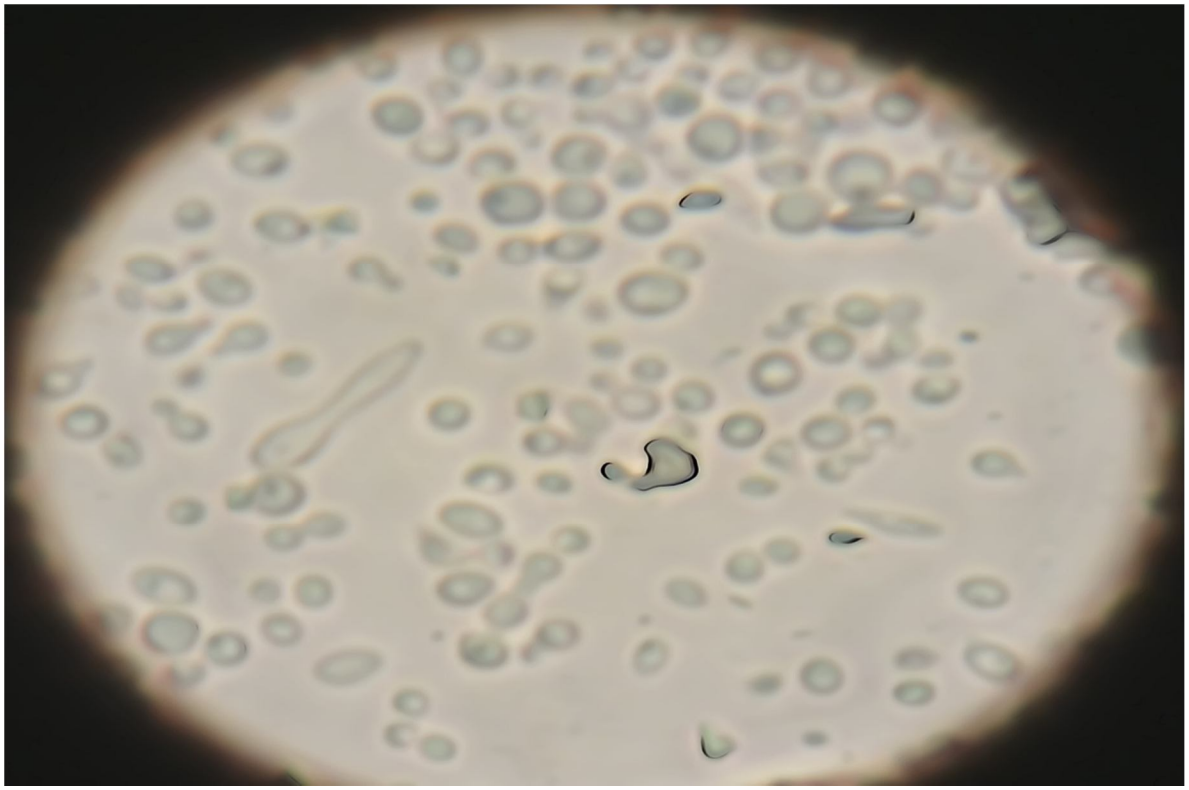
Yeast cells in LPCB mount



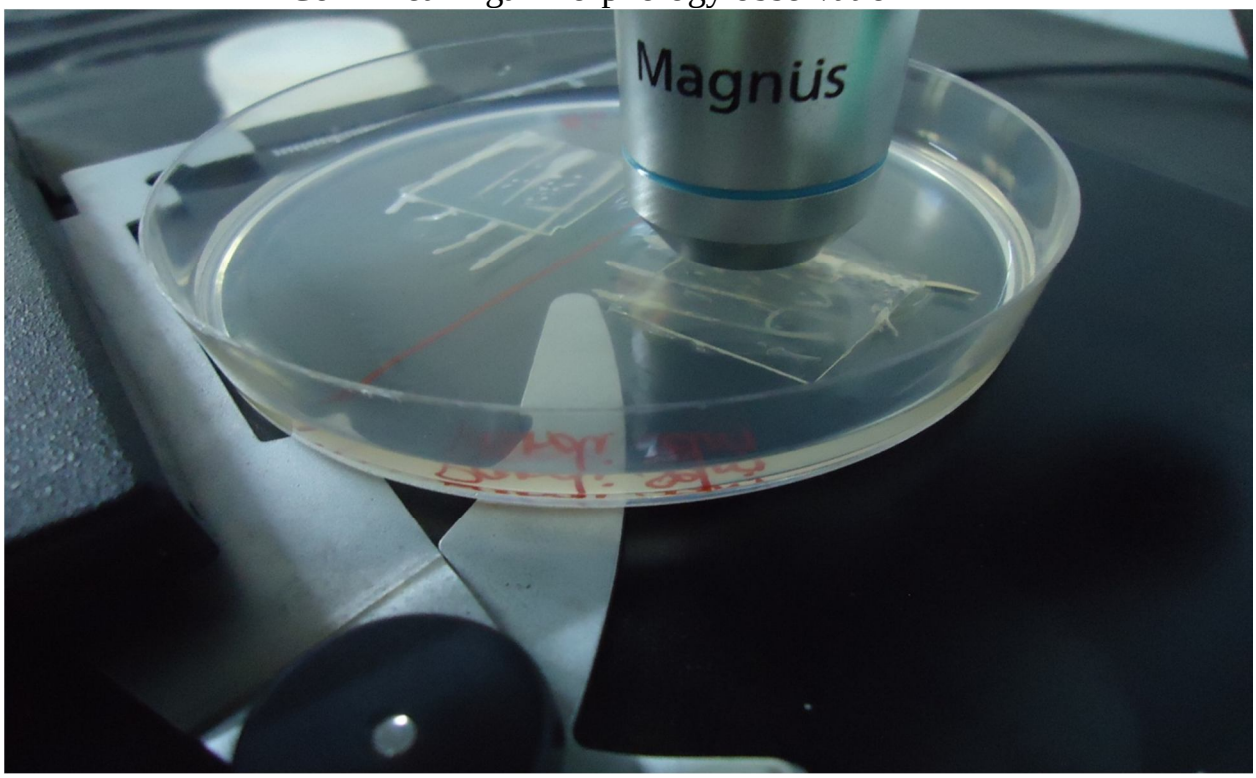
Germ Tube Test- pseudohyphea



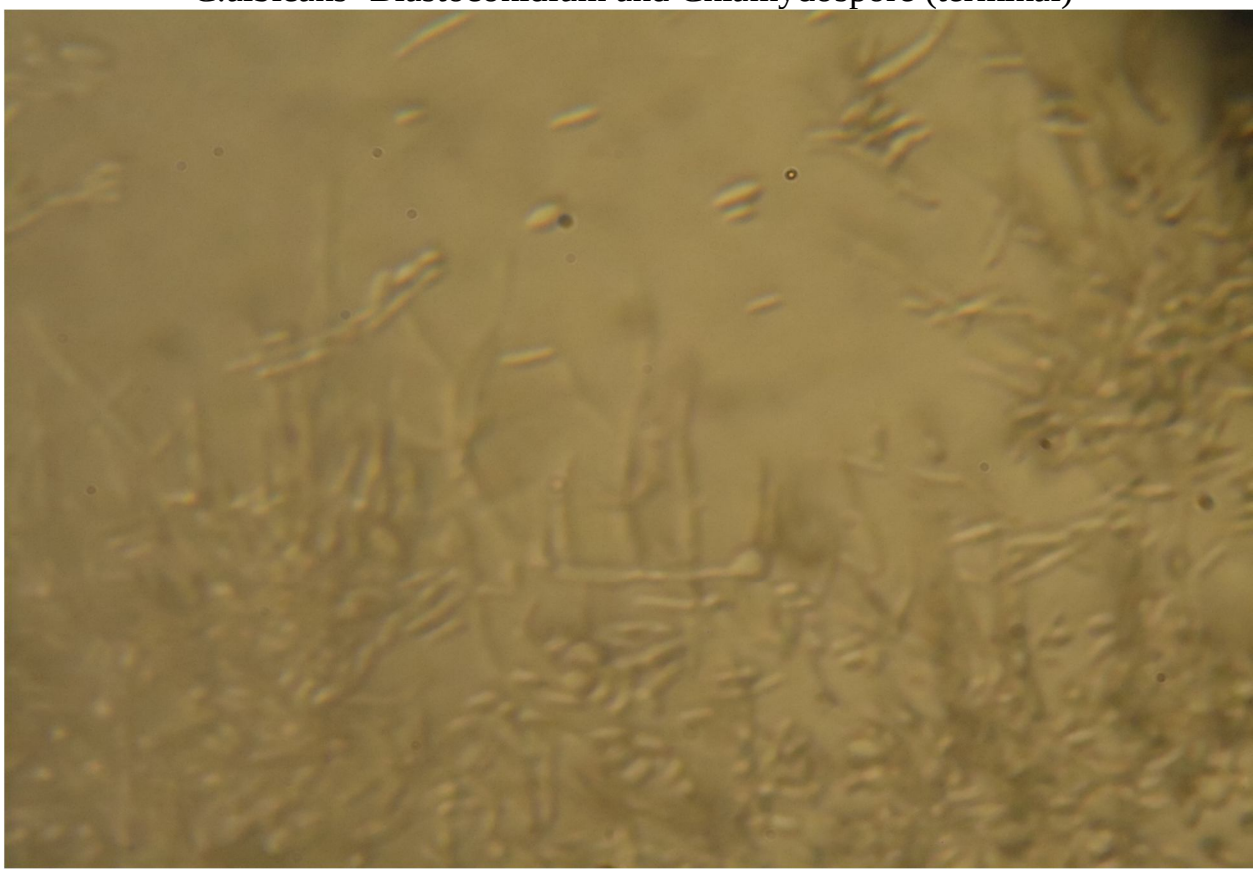
Germ Tube Test- germ tube formation by C.albicans



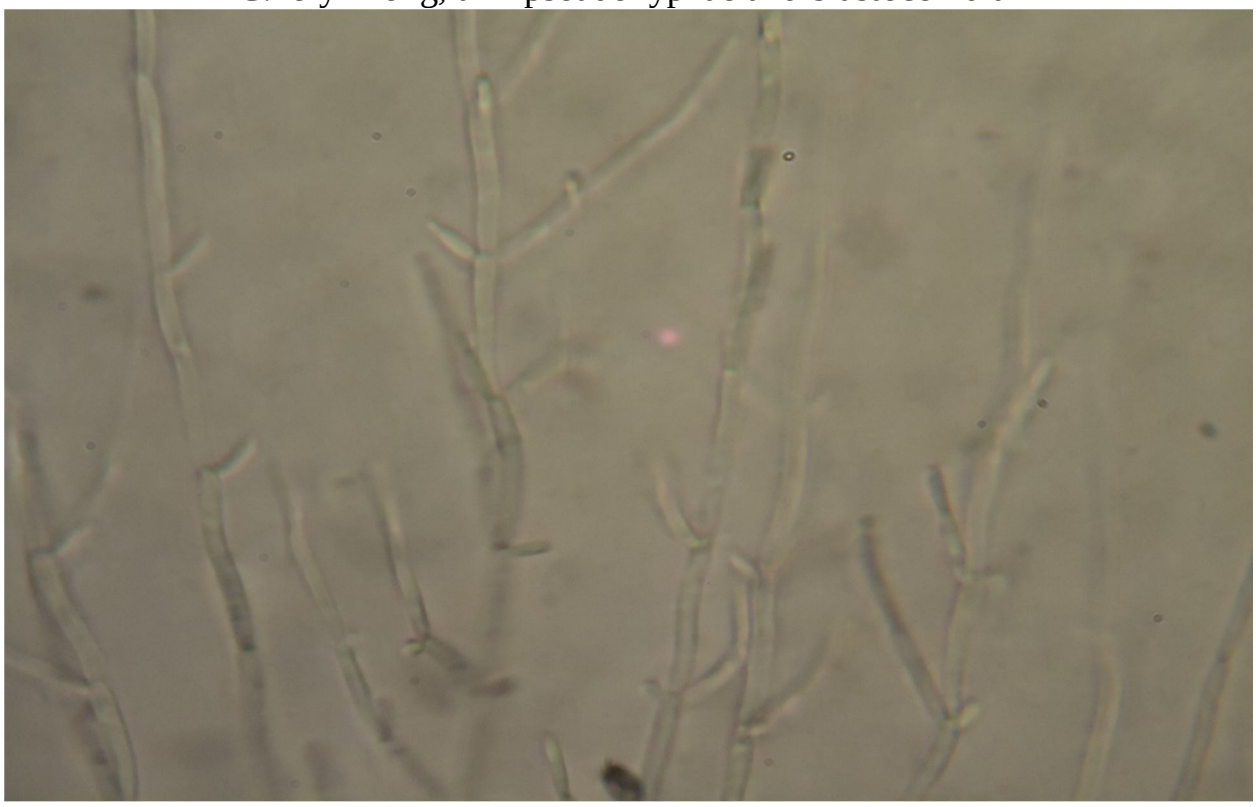
Corn Meal Agar morphology observation



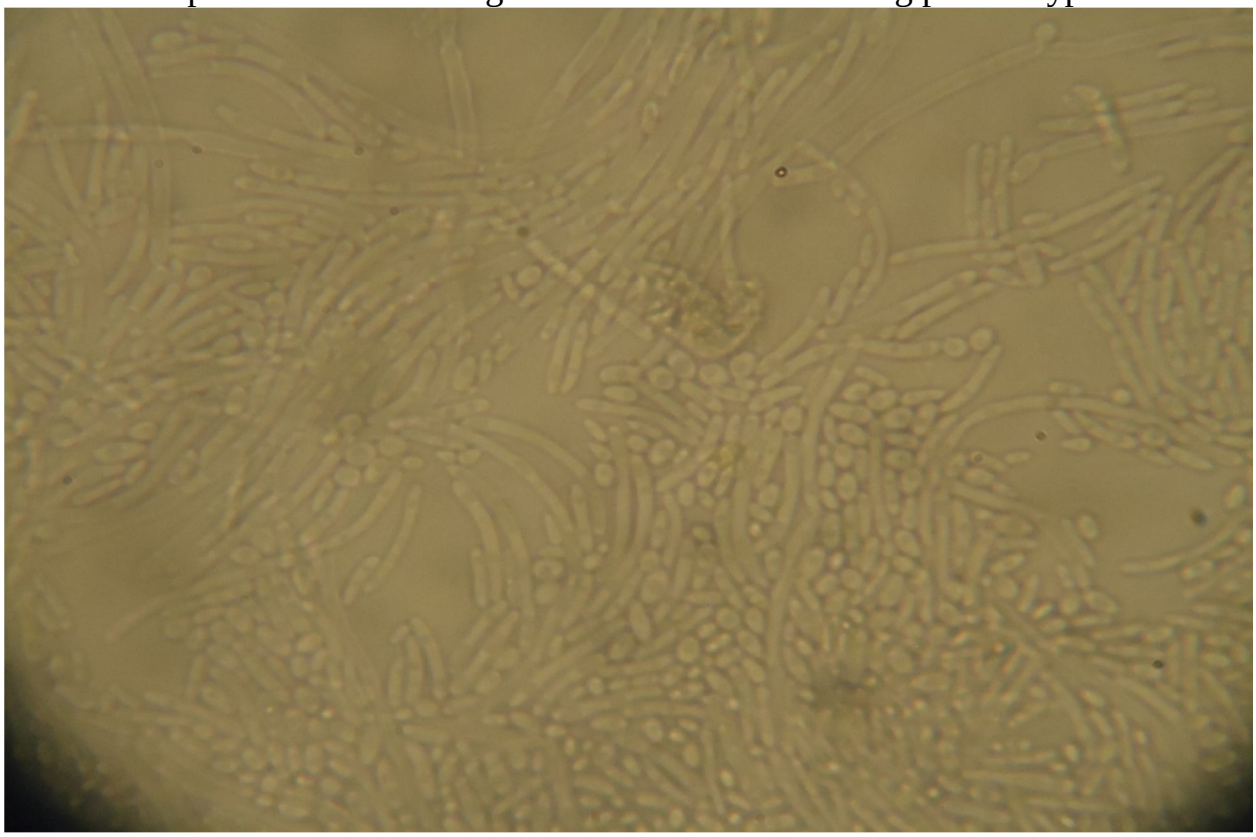
C.albicans- Blastoconidium and Chlamydospore (terminal)



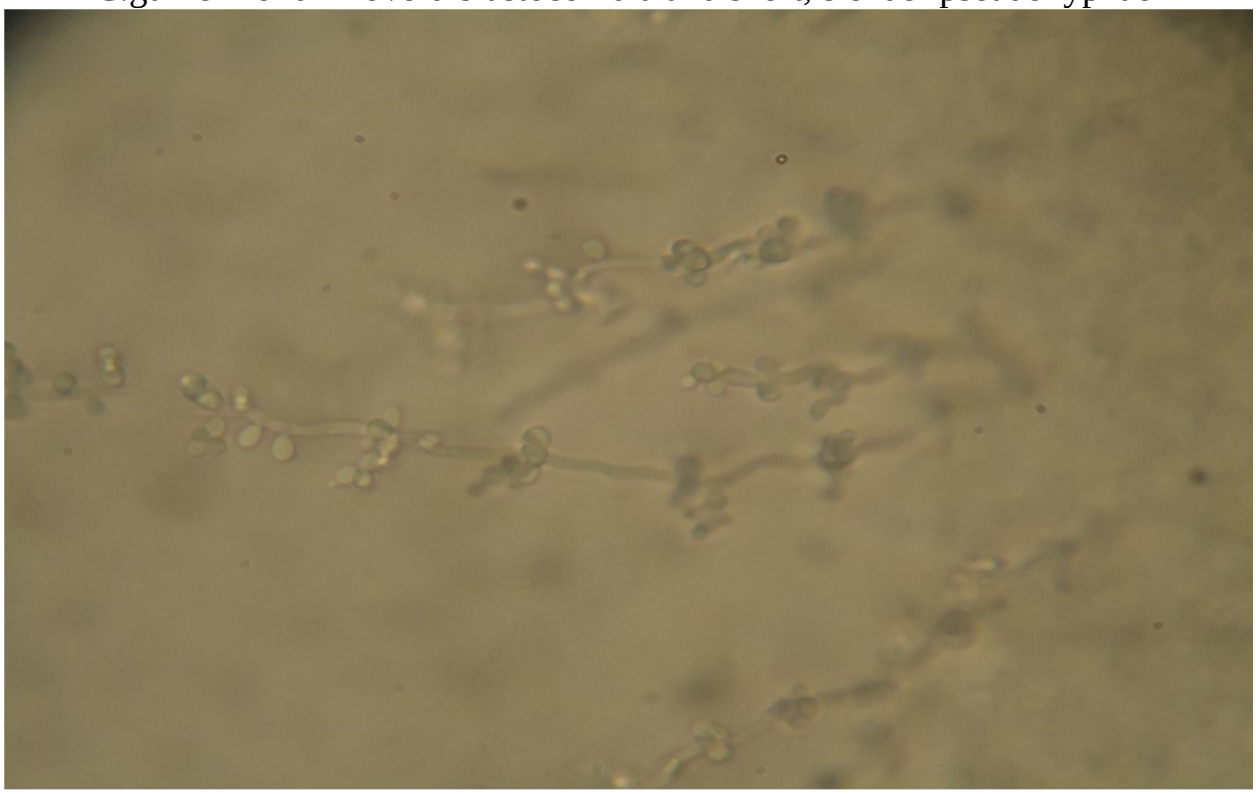
C.kefyr- long, thin pseudohyphae and blastoconidia



C.tropicalis -oval or elongated blastoconidia and long pseudohyphae

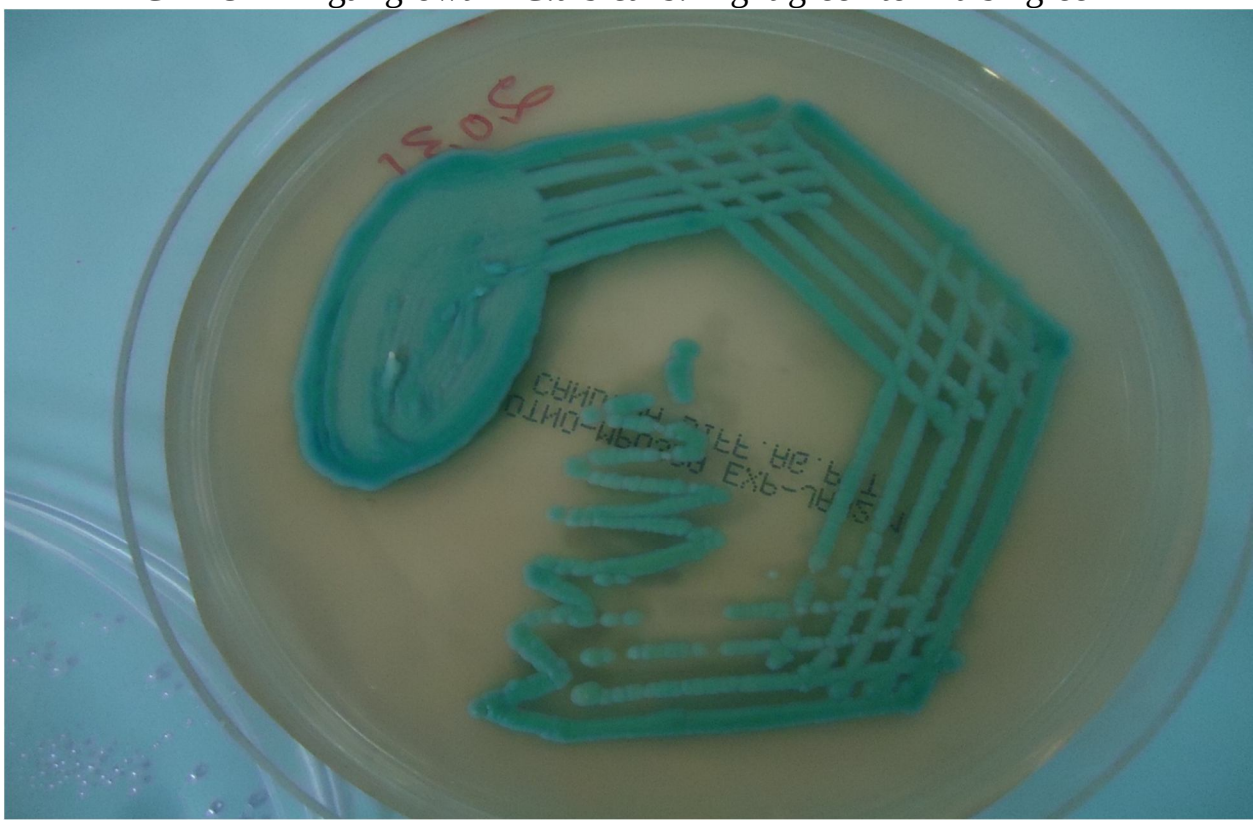


C.guillermondii - ovoid blastoconidia and short, slender pseudohyphae

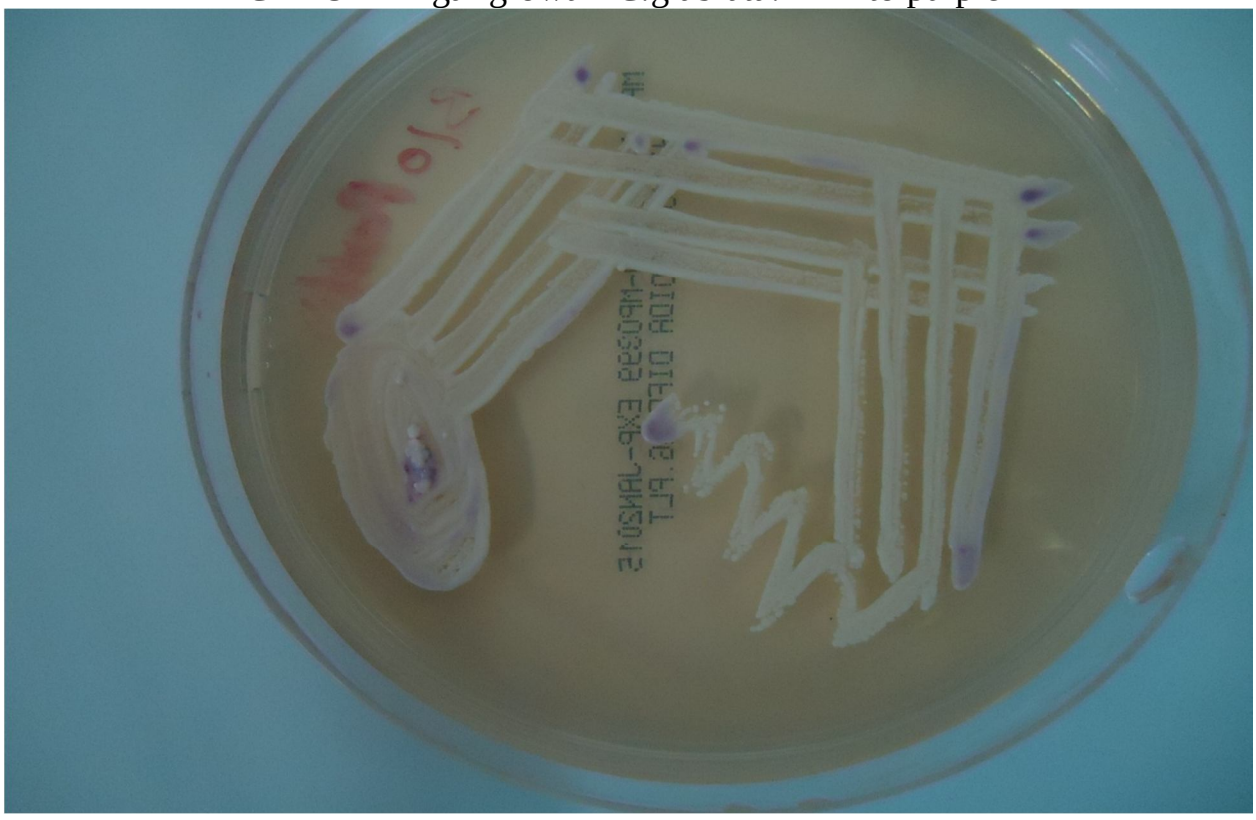


CHROMEAgar growth - color production of different specis

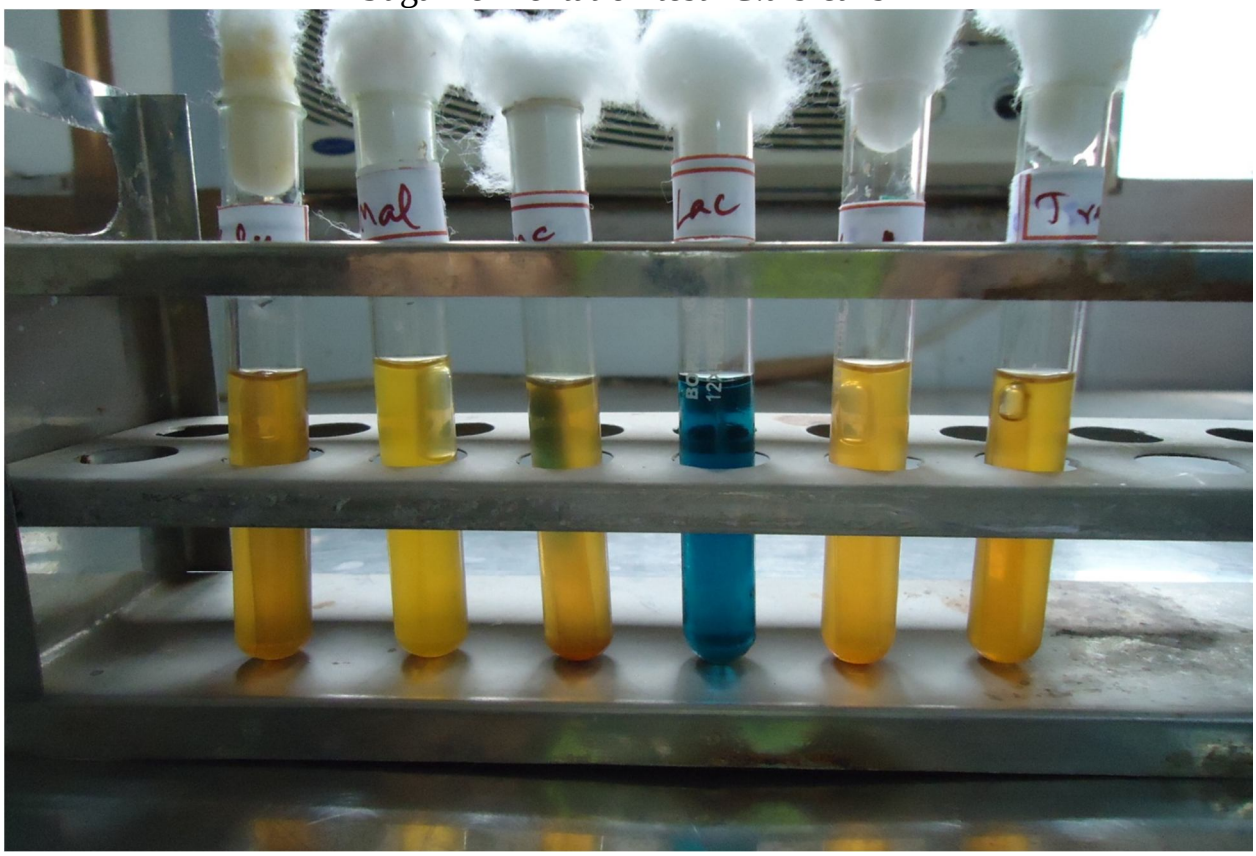
CHROMEAgar growth - C.albicans: Light green to Bluish green



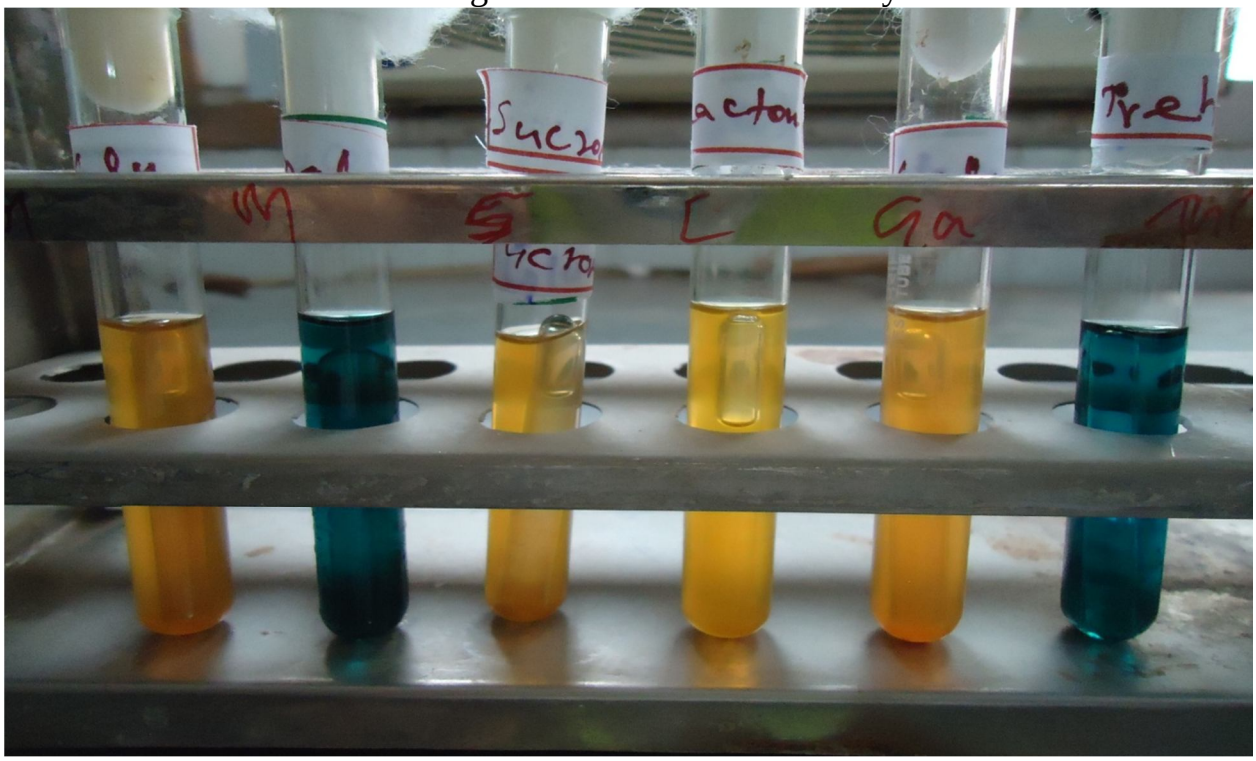
CHROMEAgar growth- C.glabrata: Pink to purple



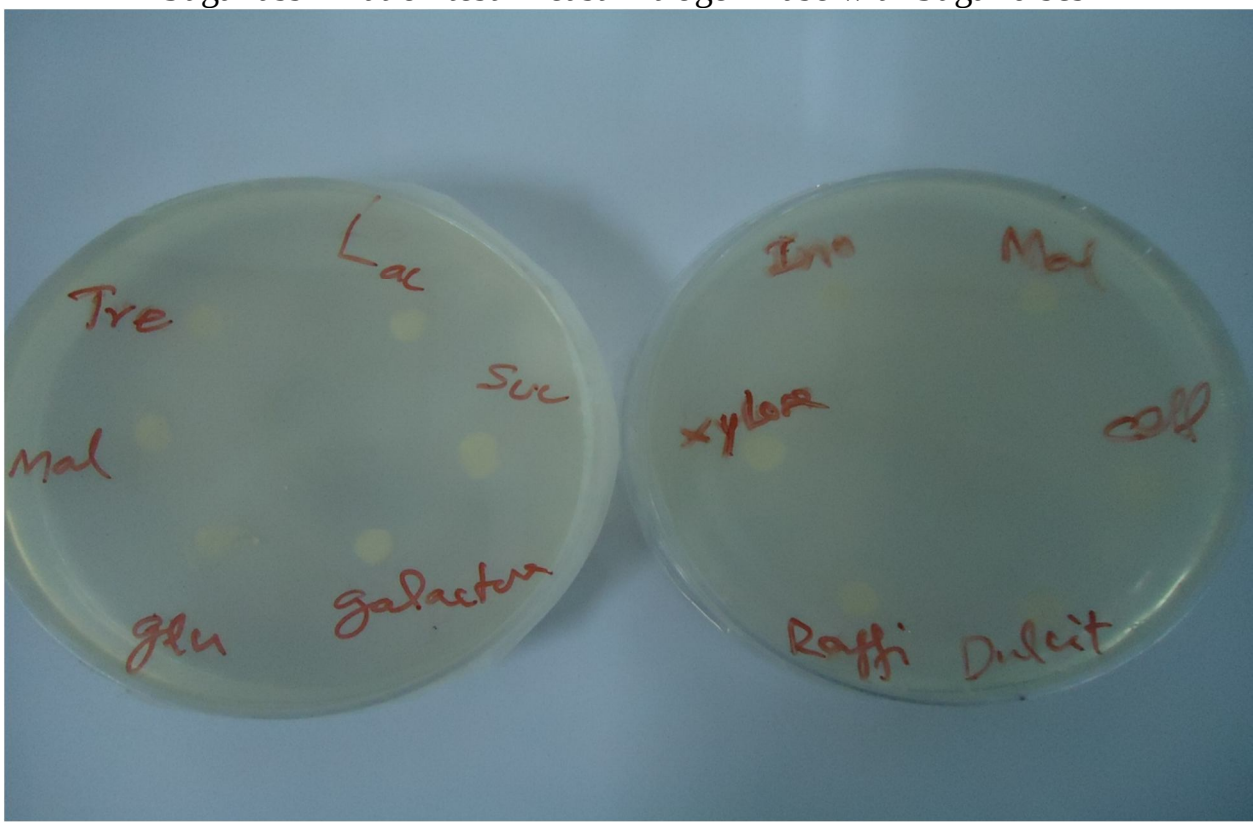
Sugar fermentation test- C.albicans



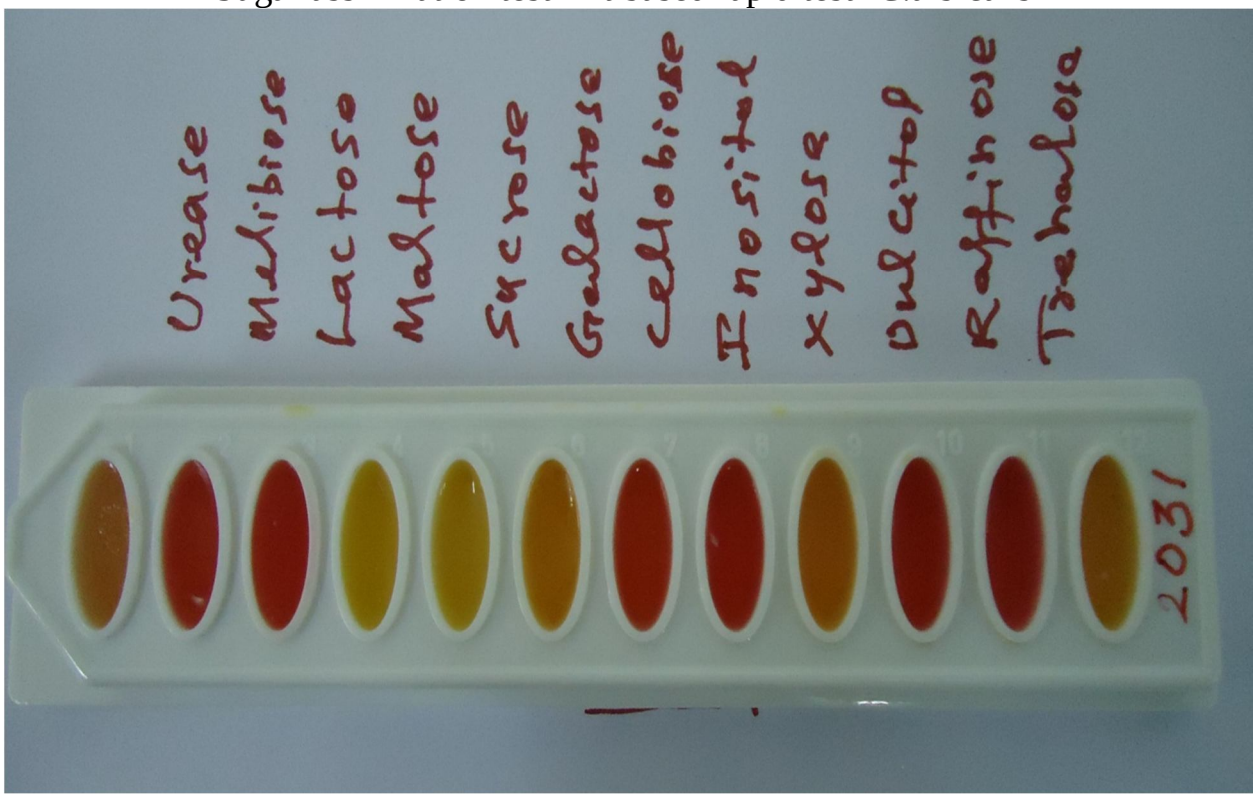
Sugar fermentation test- C.kefyr



Sugar assimilation test- Yeast Nitrogen Base with Sugar discs

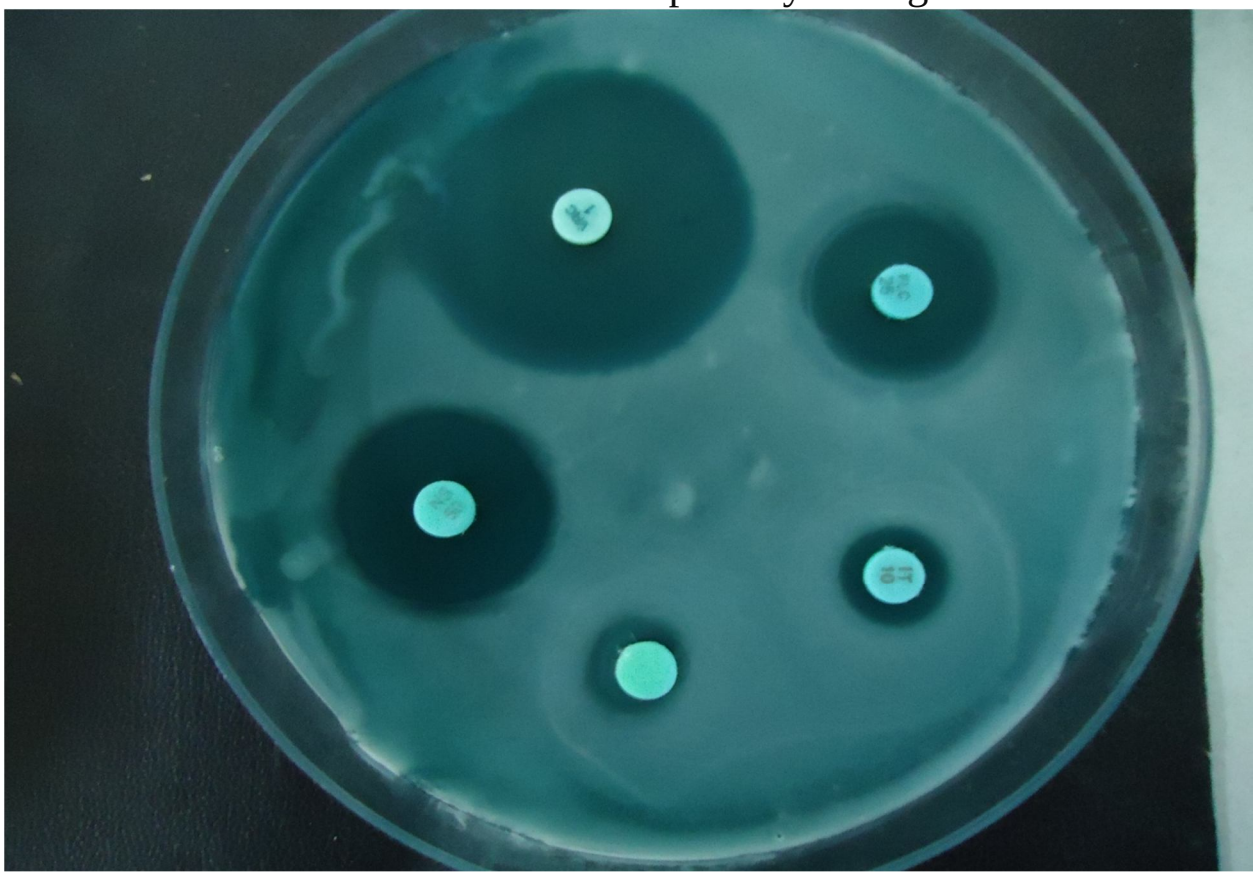


Sugar assimilation test- kit based rapid test- C.albicans

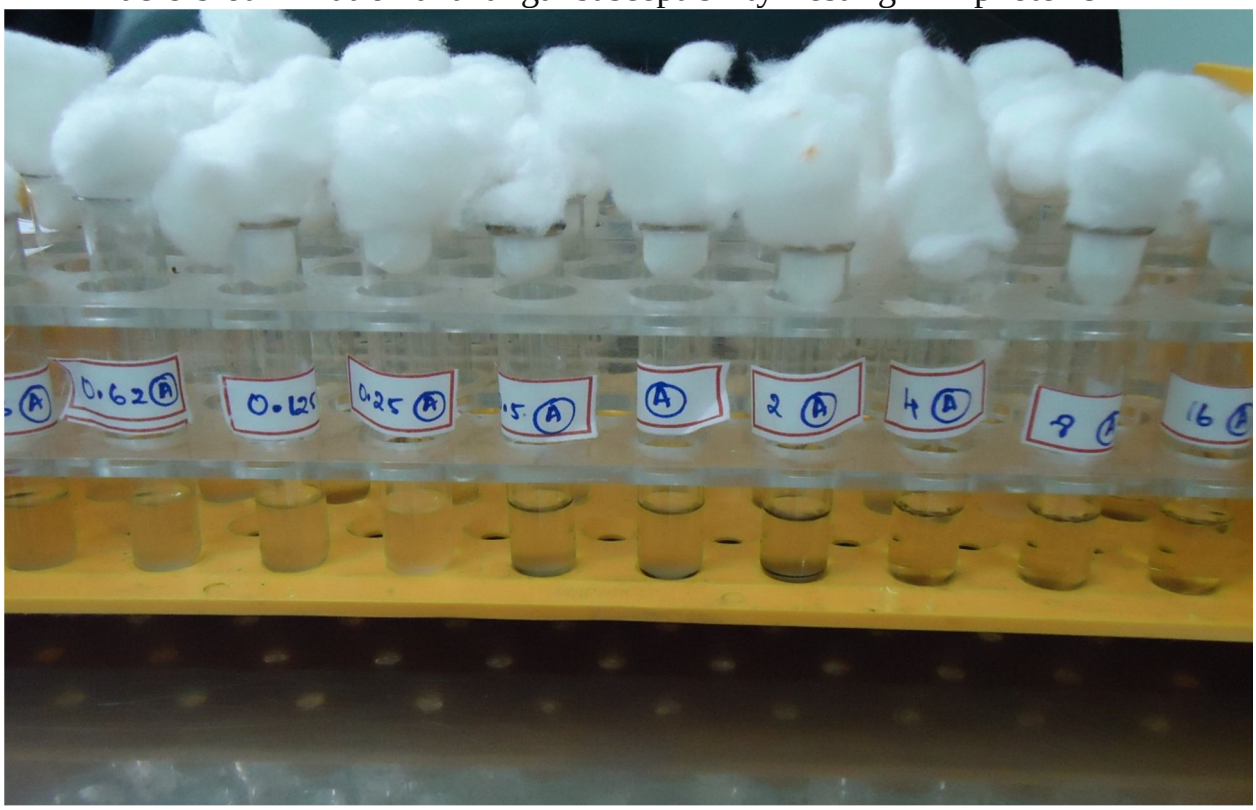


Sugar assimilation test- kit based rapid test- C.guillermondi

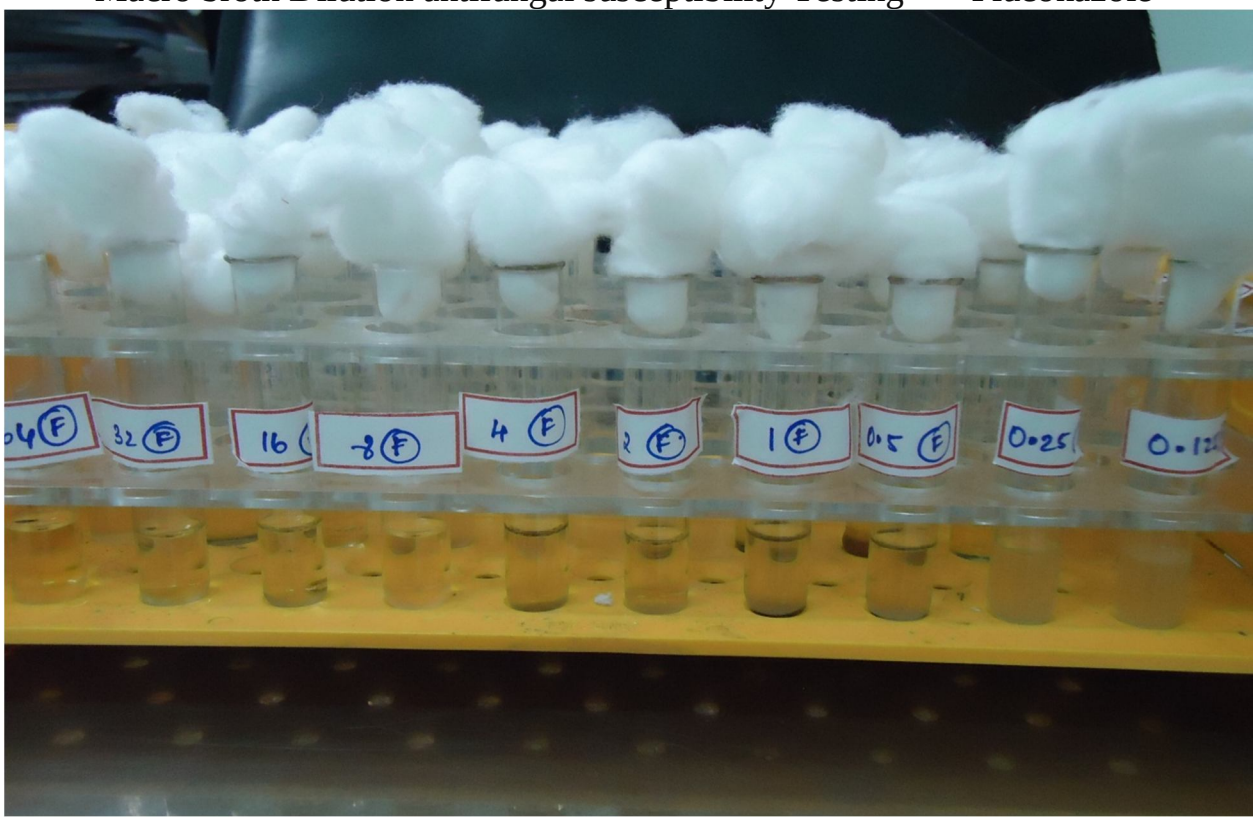
Disk diffusion susceptibility testing



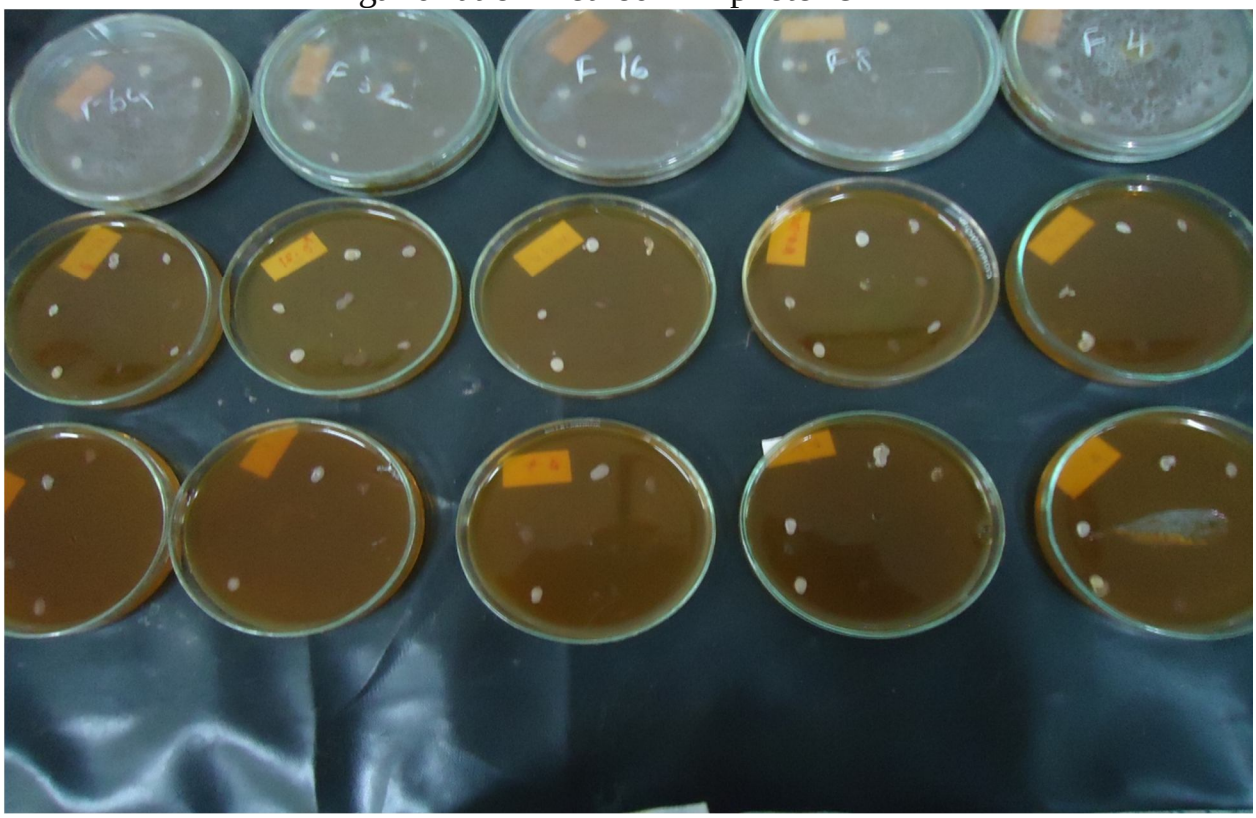
Macro broth Dilution antifungal susceptibility Testing -Amphotericin B



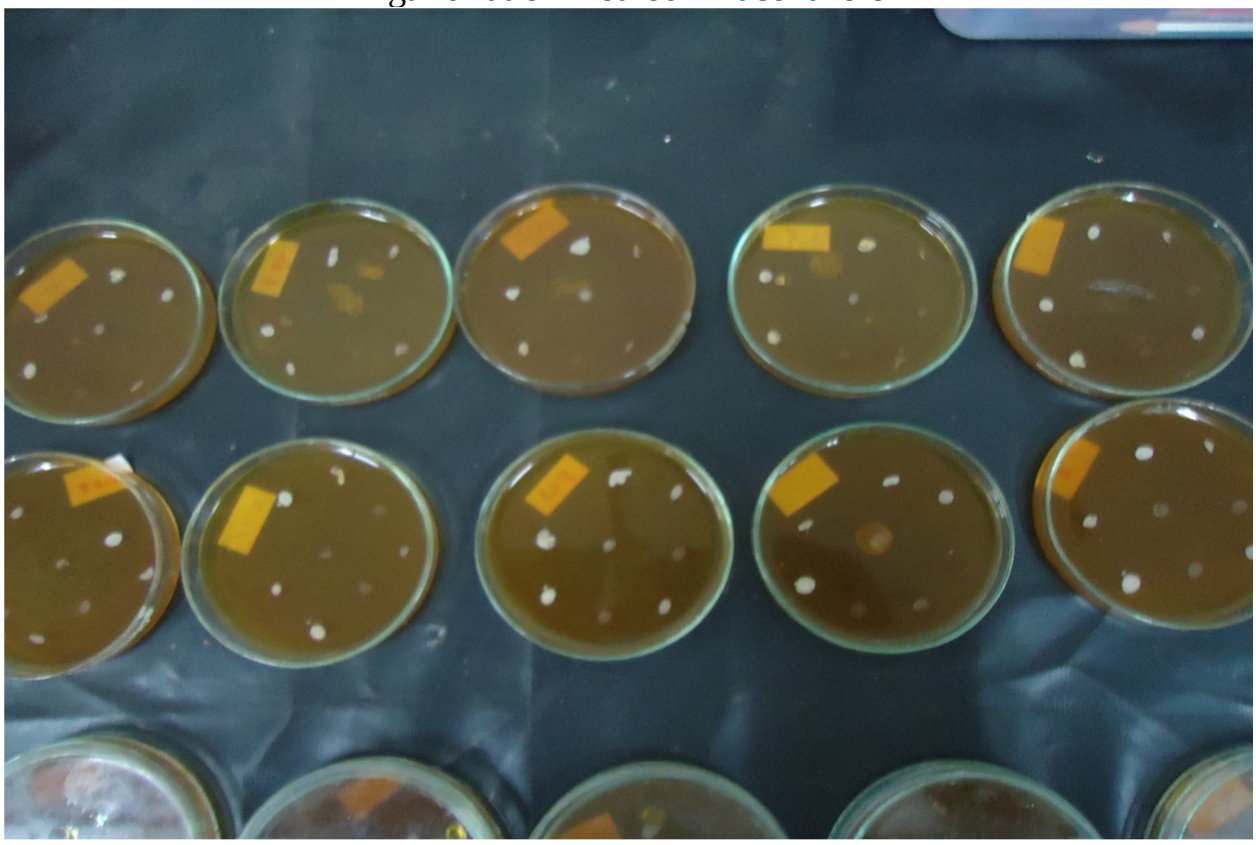
Macro broth Dilution antifungal susceptibility Testing -Fluconazole



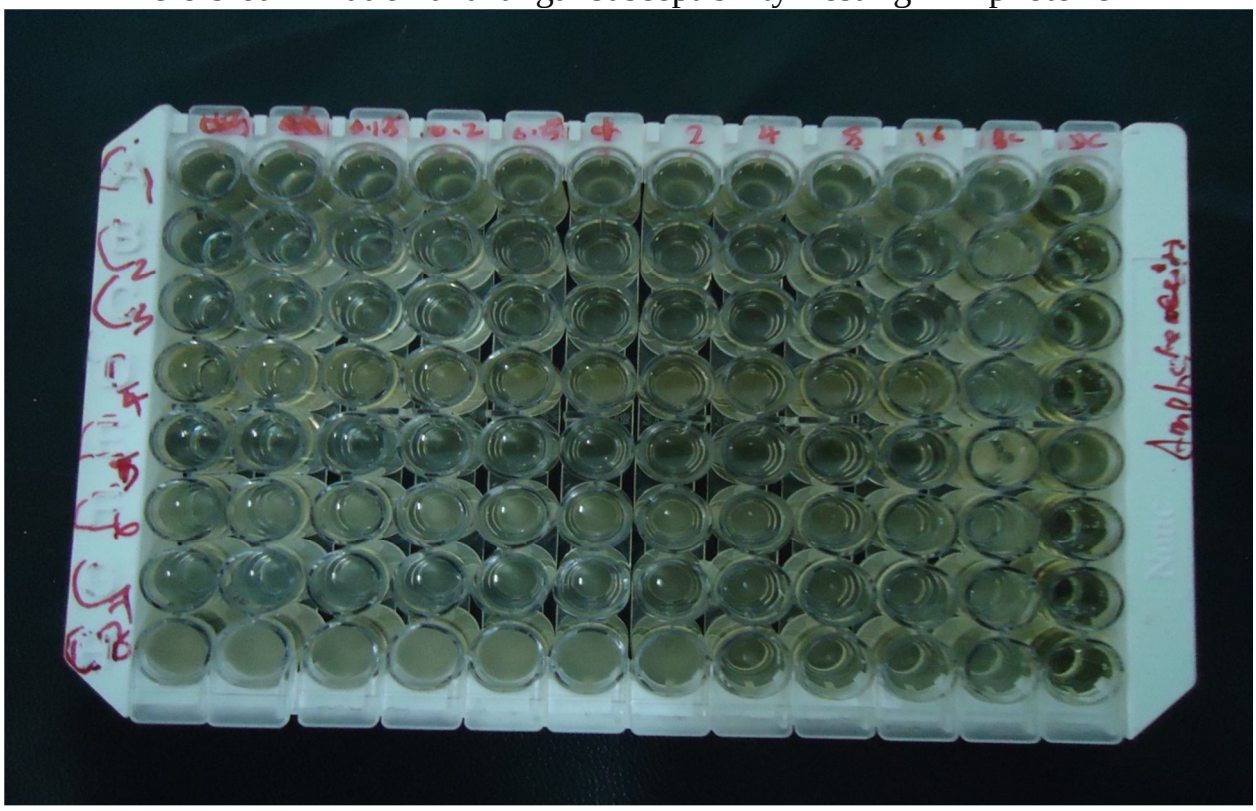
Agar dilution method- Amphotericin B



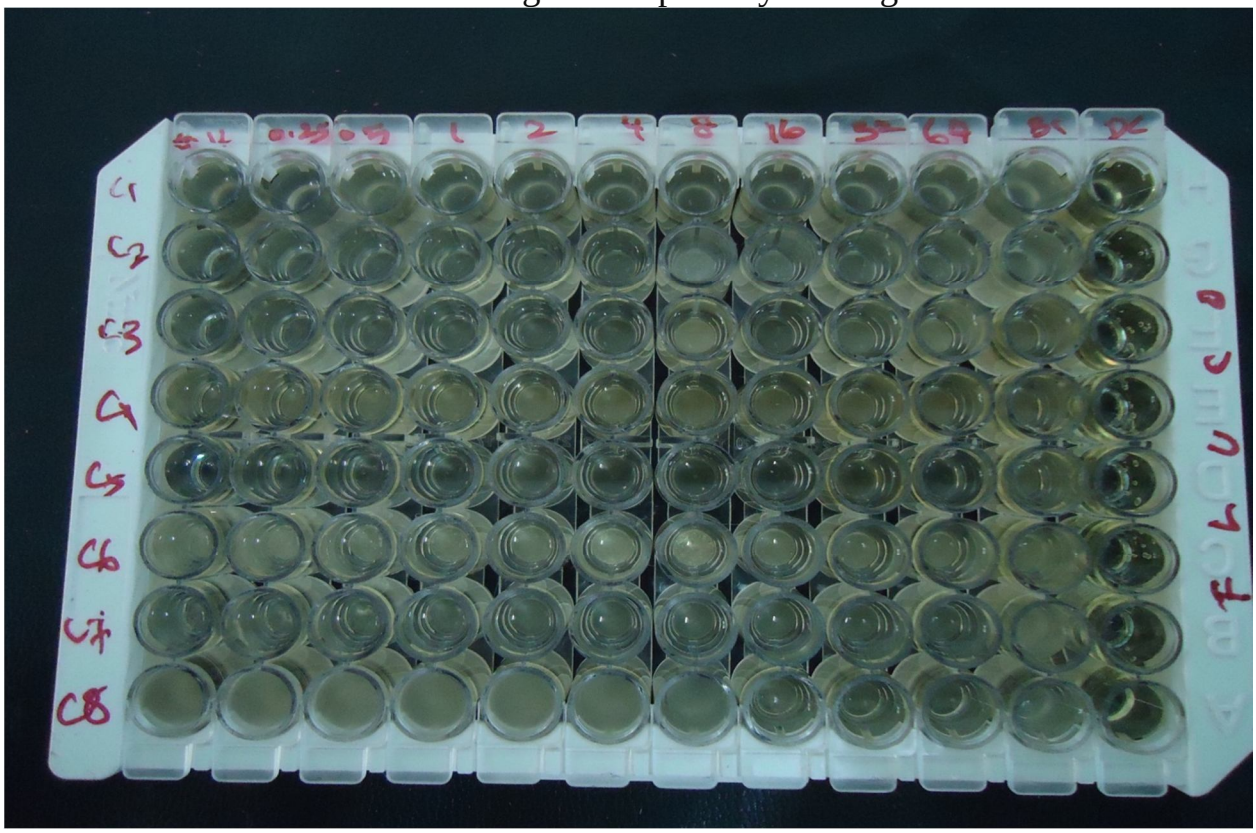
Agar dilution method- Fluconazole



Micro broth Dilution antifungal susceptibility Testing -Amphotericin



Micro broth Dilution antifungal susceptibility Testing -Fluconazole



RESULTS

RESULTS

In this study, 200 neonatal cases were selected from NICU, Institute of Child Health & Research Centre, GRH, and Madurai. The age group of neonates was those who admitted before 28 days of life.

On analyzing 200 cases of neonates, the sex distribution of selected cases was found to be male 99 (49.5%) and female 101 (50.5%). Distribution of cases in relation to sex given in Table. 1.

Table 1: Sex wise distribution of 200 selected cases.(Fig:1)

Gender	No. of cases	Percentage
Male	99	49.5%
Female	101	50.5%
Total	200	100%

The cases were further analyzed by admitted ward. Cases selected from Inborn ward (institutional delivery babies admitting unit-NICU) was 94(47%), from Sick Neonatal Nursery- SNN (sick labour theatre delivered babies, referral cases or post natal ward neonate admitting unit) was 97(48.5%) and from Pediatric Surgery Unit (PSU) was 9(4.5%). Distribution of cases in relation to ward is given in Table. 2

Table 2: Distribution of cases in relation to ward.(Fig:2)

Ward	No of Cases	Percentage
NICU	94	47%
SNN	97	48.5%
PSU	9	4.5%
Total	200	100%

The above cases were further analysed by their birth weight. There were 40(20%) cases of normal weight (N >2.5kg), 111(55.5%) cases of low birth weight (LBW <2.5 kg), 41(20.5%) cases of very low birth (VLBW <1.5 kg) and 8(4%) cases of Extremely low birth (ELBW <1.0 kg). Distribution of cases in relation to birth weight given in Table. 3.

Table 3: Distribution of cases in relation to birth weight. (Fig:3)

Birth weight	No of Cases	Percentage
Normal	40	20%
LBW	111	55.5%
VLBW	41	20.5%
ELBW	8	4%
Total	200	100%

Maturity at birth of each neonates were categorized into term and preterm (below 37 weeks). Term neonates were 85(42.5%) and preterm neonates were 115(47.5). Among the 115 preterm babies, 31(15.5%) cases were late preterm (>34 weeks), 62(31%) cases were preterm between 30-34 weeks and 22(11%) cases preterm below 30 weeks.

Table 4: Distribution of cases in relation to maturity. (Fig:4)

Maturity	No of cases	Percentage
Term	85	42.5%
Preterm 34-37 weeks	31	15.5%
Preterm between 30-34 weeks	62	31%
Preterm below 30 weeks	22	11%
Total	200	100%

Various categories of risk factors were analyzed in this study. About 78 (39%) cases had stayed more than 7 days in wards at the time of sample collection. Preterm with VLBW and ELBW, sepsis proven by bacterial culture and post operative neonates were mainly kept in the ward for prolonged duration.

Mechanical ventilator support was given for 47 (28.5%) cases. All the neonates who received surfactant therapy were put on ventilator. Neonates with respiratory Distress syndrome (RDS), with severe sepsis and some cases of post operative care were supported with mechanical ventilation.

Umbilical catheter was inserted to 13 (6.5%) neonates, who underwent exchange blood transfusion and it was done for neonatal jaundice. No neonate had central venous catheter insertion.

Surfactant therapy was administered for 20(10%) cases, mostly to the VLBW and ELBW neonates.

Among the 200 selected cases 45(22.5%) cases were transfused with blood Fresh Frozen Plasma (FFP) and platelet. Apart from exchange blood transfusion for jaundice ,all other transfusions were received by most of the neonatal sepsis babies as a therapeutic measure.TPN was not used for any of the cases.

Antibiotics were started initially to all the admitted babies; it was continued beyond 5 days for suspected sepsis or proven sepsis cases and post operative neonates. Out of the200 selected neonates , 87(43.5) cases received broad spectrum antibiotics beyond 5 days.

About 9 cases (4.5%) underwent major surgical intervention and they were in post operative care.

Table 5: Risk factors among selected cases(Fig:5)

S. No	Risk factors	Number of cases
1.	Hospital stay > 7 days	78
2.	Mechanical ventilation support	47
3.	H/O umbilical line insertion	13

4.	Surfactant therapy	20
5	H/O Blood transfusion	43
6.	Broad spectrum antibiotics administration for 5 days	87
7.	Underwent major surgery	9

Sepsis screening were noted down at the time of sample collection. Total WBC count, I/T ratio, platelet count and CRP were routinely done for suspected neonatal sepsis cases. The results were analyzed and this is tabulated in table - 6.

Table 6: Sepsis screening reports. (Fig:6)

S. No	Parameters	Number of positive cases
1.	WBC count <7500 or >40000 cells/cmm	44
2.	I/T ratio >0.2	133
3.	Platelet count < 1,00,000 cells/cmm	45
4.	CRP positive	130

There were 103 (51.5%) culture proven cases for bacterial sepsis, among the screened neonates. Among that, 37 cases were EOS and 66 cases were LOS.

Sample like blood 179 (89.5%), CSF 11(5.5%) or urine 10 (5%) were collected from all selected cases.

Table 7:Distribution of types of sample. (Fig:7)

S. No	Type of sample	No of samples	Percentage
1.	Blood	179	89.5%
2.	CSF	11	5.5%
3.	Urine	10	5%
4.	Total	200	100%

Out of 11 samples only one CSF sample showed presences of yeast cells in wet mount and Gram staining . India ink preparation did not show capsular structure from any of the CSF samples.

Out of 200 samples inoculated on to SDA, 13 samples showed growth. In that 12 were from blood sample and 1 from CSF sample. All urine samples were culture negative for fungus.

Table 8: Distribution of observed positive fungal growth. (Fig:8)

S. No	Type of sample	No of samples inoculated to SDA	Positive fungal growth observed
1.	Blood	179	12
2.	CSF	11	1
3.	Urine	10	0

4.	Total	200	13

On macroscopic observation, all the 13 growth were cream colored, mucoid and pasty colonies. On microscopic observation of the growth by the wet mount preparation, Gram staining and LPCB mount showed budding yeast cells which was Gram positive. In India ink preparation no capsular structure was demonstrated from any of the growth. From the above methods, all of the growths were identified presumptively as Candida species.

Speciation of candida :

Germ tube test were positive in 4 isolates out of 13 growths. All this isolates were reinoculated in SDA and incubated at 45°C for further speciation. After 24-48hrs growth were observed in 3 isolates, which were marked as C.albicans and no growth in one isolate which was C.dubliniensis.

The growth morphology of all isolates in Corn meal agar was examined under microscope for the formation of blastoconidia, chlamydospore and their arrangement and for the production of pseudohyphae. The above observations were compared with growth patterns described for Candida species.

In CHROMEagar, color production of each candida species were noted and according to the color, species of Candida were identified. Speciation of candida done by special methods is given in Table 9.

Biochemical reactions like sugar fermentation and sugar assimilation were done and results were tabulated in table No 10.

From above special methods and biochemical test, all 13 isolates of candida were differentiated to species level, as C.albicans 3 in number, C.guilliermondii 3 in number, C.glabrata 2 in number and C.parapsilosis, C.dubliniensis, C.tropicalis, C.krusei, C.kefyr each one in number. In that 12 were isolated from blood samples and one C.albicans was from CSF. Species level distribution of Candida is given in table 11.

Table 9: Speciation by special methods.

Candida species	GTT	Growth at 45°C	CMA morphology	CHROMEagar growth	Isolate No
C.albicans	+	+	Irregular or spherical clusters of blastospores at septa. Numerous chlamydospores single or in clusters, Terminal chlamydospores	Light green to Bluish green	C-1, C-4, C-6
C.parapsilosis	-	-	Fine and coarse mycelium (gaint forms). Blastospores single or in short chains at septa or distal ends of cells	Cream/off-white	C-2
C.dubliniensis	+	-	Irregular or spherical clusters of blastospores at septa. Numerous chlamydospores single or in clusters	Dark green	C-3
C.glabrata	-	-	Very elongate cells which readily fall apart and lie parallel. "Logs in stream" appearance, not	Pink to purple, glorry	C-5, C-13

			produce pseudohypha		
C.guilliermondii	-	-	Very fine mycelium. Small clusters of blastospores at the septa	White to cream	C-7 C-11, C-12
C.tropicalis	-	-	Blastospores anywhere along mycelium or in irregular clusters. Chlamydospores very rare	Blue with pink halo	C-8
C.krusei	-	-	Elongate cells forming a branched mycelium easily disintegrated. "Crossed sticks" of septa.	Spreading pink	C-9
C.kefyr	-	-	Very elongated cells, abundance of pseudohyphae	White	C-10

GTT- Germ Tube Test, CMA - Corn Meal Agar, + = Positive Reaction, - = Negative Reaction.

Table No 10: Biochemical reactions - sugar fermentation and sugar assimilation

Candida species	Assimilation of :												Fermentation of:						Isolate No
	Glu	Mal	Suc	Lac	Gal	Mel	Cel	Ino	Xyl	Raf	Tre	Dul	Glu	Mal	Suc	Lac	Gal	Tre	
C.albicans	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	A	-	AG	AG	C-1, C-4, C-6
C.parapsilsis	+	+	+	-	+	-	-	-	+	-	+	-	AG	-	-	-	-	-	C-2
C.dubliniensis	+	+	+	-	+	-	-	-	+	-	+	-	AG	AG	-	-	AG	AG	C-3
C.glabrata	+	+	-	-	-	-	-	-	-	-	+	-	AG	-	-	-	-	AG	C-5, C-13
C.guillermondi	+	+	+	-	+	+	+	-	+	+	+	+	AG	-	AG	-	AG	AG	C-7 C-11, C-12

C.tropicalis	+	+	+	-	+	-	+	-	+	-	+	-	AG	AG	AG	-	A G	AG	C-8
C.krusei	+	-	-	-	-	-	-	-	-	-	-	-	AG	-	-	-	-	-	C-9
C.kefyr	+	-	+	+	+	-	+	-	+	+	-	-	AG	-	AG	AG	A G	-	C-10

Notes : Glu = Glucose, Mal = Maltose, Suc = Sucrose, Lac =Lactose, Cel = Cellobiose, Gal = Galactose, Tre = Trehalose, Raf = Raffinose, Mel = Melibiose, Xyl = Xylose, Ino = Inositol, Dul = Dulcitol; + = Positive Reaction, - = Negative Reaction, V = Variation, A = Acid Production, G = Gas Production.

Table 11: Species level distribution of Candida(Fig:9)

S.No	Name of the species	Number of isolates	Percentage
1	C.albicans	3	23%
2	C.guillermondi	3	23%
3	C.glabrata	2	16%
4	C.krusei	1	7.6%
5	C.parapsilosis	1	7.6%
6	C.tropicalis	1	7.6%
7	C.kefyr	1	7.6%
8	C.dubliniensis	1	7.6%
	Total	13	100%

Table 12: Sex wise distribution of fungal sepsis cases. (Fig:10)

S. No	Sex	No of Fungal Sepsis Cases	Percentage
1	Male	7	54%
2	Female	6	46%
3	Total	13	100%

Out of 13 fungal pathogen 7(54%) were recovered from male neonates.. Remaining 6(46%) were female neonates.

Fungal sepsis neonates were analyzed according to place of birth Among 13 cases, 8 (62%) were out born cases. Distribution of positive cases in relation to place of birth is given in Table 13.

Table 13: Distribution of positive cases in relation to place of birth. (Fig:11)

S. No	Place of birth	No. of Fungal Sepsis Cases	Percentage
1	Inborn	5	38%
2	Outborn	8	62%
3	Total	13	100%

Low birth weight neonates showed higher incidence of infection than normal birth weight neonates. A total of 10 cases of LBW were affected by fungal sepsis and in that 5 of them were VLBW and 1 was ELBW neonates.

Table 14. Distribution of positive cases in relation to birth weight. (Fig:12)

S. No	Birth Weight	No. of Fungal Sepsis cases	Percentage
1	Normal	3	23%
2	LBW <2500gms	4	31%
3	VLBW <1500gms	5	38.4%
4	ELBW	1	7.6%

There were more incidence of sepsis in preterm neonates. About 9 cases were in preterm category. Among that, one was < 30 weeks of gestational age and three were late preterm > 34 weeks.

Table 15: Distribution of fungal sepsis cases in relation to maturity.(Fig:13)

S. No	Maturity	No. of Fungal Sepsis cases	Percentage
1	Term	4	31%
2	Preterm >34 weeks	3	23%
3	Preterm between 30-34 weeks	5	38.4%
4	Preterm below 30 weeks	1	7.6%

Risk factors among fungal sepsis neonates were analyzed and tabulated in table No. 16.

Septic screening reports which was noted down at the time of sample collection was correlated with positive cases. There were 10 cases with reduced platelet count < 100000 cells/cmm and 9 cases of total count < 7500 cells/cmm. I/T ratio more than 0.3 and raised CRP positive above 1.0 mg / dl were seen in all 13 reports.

Table 16: Risk factors associated with fungal sepsis cases.(Fig:14)

S. No	Risk factors	Number of cases associated with fungal sepsis
1.	Hospital stay > 7 days	7
2.	Mechanical ventilation support	6
3.	H/O umbilical line insertion	1
4.	Surfactant therapy	1
5.	Broad spectrum antibiotics administration for more than 5 days	8

Table 17:Sepsis screening reports associated with fungal sepsis.(Fig:15)

S. No	Parameters	Number of cases associated with fungal sepsis	Percentage
1.	WBC count <7500 or >40000 cells/cmm	9	69%
2.	I/T ratio >0.3	13	100%
3.	Platelet count < 1,00,000 cells/cmm	10	77%
4.	CRP positive above 1.0 mg / dl	13	100%

Out of 200 cases, 103 cases were positive for bacterial culture and they were treated with broad spectrum antibiotics. Among the 103 cases 10 cases showed fungal growth from their samples. According to onset of sepsis, 37 were noted as EOS and 66 were LOS. Of the 10 fungal positive cases, 3 were from EOS and 7 were LOS. This increased incident in fungal sepsis among LOS cases might be due to prolonged hospital stay and prolonged antibiotic treatment.

Table 18: Distribution of fungal sepsis cases in relation to culture proven for bacterial sepsis cases

S. No	Culture proven for bacterial sepsis cases		No. of Fungal Sepsis cases
1.	EOS	37 (36%)	3 (2.7%)
2.	LOS	66 (64%)	7 (6.7%)
3.	Total	103 (100%)	10 (9.4%)

Antifungal susceptibility testing was done for isolated fungal pathogen. The methods adapted for antifungal susceptibility testing were Macro broth and Micro broth Dilution method, Agar dilution method and disk diffusion method. For the first three methods only Amphotericin B and Fluconazole were used. For disk diffusion method Itraconazole, Voriconazole and Nystatin were also tested. The results were observed as per standard guidelines given by CLSI.

Antifungal susceptibility test interpretation results given in table No.19.

Table No 19: Interpretation of antifungal susceptibility.

S. No	Candida species	Macro broth dilution, Micro broth dilution and Agar dilution method				Disk diffusion method									
		AMB MIC (µg/ml)	I	FLU MIC (µg/ml)	I	AMB ZD	I	FLU ZD	I	ITR ZD	I	VOR ZD	I	NYS ZD	I
C1	C.albicans	0.25	S	64	R	10	SDD	26	S	14	SDD	36	S	22	S
C2	C.parapsilsis	0.125	S	4	SDD	17	S	15	SDD	16	SDD	34	S	27	S
C3	C.dubliniensis	1.0	S	2	S	15	SDD	17	SDD	16	SDD	27	S	20	SDD
C4	C.albicans	16	R	64	R	9	R	13	R	12	R	14	SDD	25	S
C5	C.glabrata	0.125	S	64	R	10	S	8	R	26	S	31	S	19	SDD
C6	C.albicans	0.5	S	1.0	S	14	SDD	24	S	16	SDD	35	S	16	R
C7	C.guillermondi	0.25	S	0.5	S	17	S	20	S	16	SDD	25	S	25	S

C8	C.tropicalis	2	R	64	R	8	R	8	R	11	R	27	S	13	R
C9	C.krusei	16	R	-	-	9	R	-	-	-	-	-	-	22	S
C10	C.kefyr	0.25	S	64	R	17	S	9	R	12	R	37	S	29	S
C11	C.guillermondi	0.25	S	0.25	S	18	S	25	S	17	S	40	S	29	S
C12	C.guillermondi	0.5	S	1.0	S	20	S	26	S	14	SDD	36	S	28	S
C13	C.glabrata	1.0	S	8	S	10	SDD	20	S	22	S	37	S	26	S

MIC- Minimum Inhibitory Concentration, ZD- Zone Diameter in millimeter, I - Interpretation, S - sensitive, R - Resistant, S-DD -Susceptible Dose Dependent, AMB- Amphotericin B, FLU- Fluconazole, ITR- Itraconazole, VOR-Voriconazole, NYS-Nystatin.

Susceptible Dose Dependent was taken as sensitive.

Table No 20: Antifungal susceptibility pattern.(Fig:16)

S. NO	Antifungal agents	Number of isolates tested	Sensitive(%)	Resistant(%)
1	Amphotericin B	13	10(77%)	3(23%)
2	Fluconazole	12	8(70%)	4(30%)
3	Itraconazole	12	10(83%)	2(17%)
4	Voriconazole	12	12(100%)	0
5	Nystatin	13	11(85%)	2(15%)

Analyzing the Antifungal susceptibility pattern, Amphotericin B resistance was noticed in three isolates (23%) and remaining 10 (77%) isolates were susceptible. Out of 3, one C.albicans was resistant to Amphotericin B and C.tropicalis, C.krusei also resistant to Amphotericin B. Azole drugs tested only in 12 isolates as C.kursei have intrinsic resistance to Azoles. Fluconazole resistance was noticed in 4 isolates(30%). One C.glabrata and one C.albicans, C.tropicalis and C.kefyr showed resistance to fluconazole. Itraconazole resistance was noticed in 2 isolates (17%).One was C.albicans and other one was C.tropicalis. All 12 Candida species(100%) were sensitive for voriconazole and among them one C.albicans was in Susceptible Dose Dependent (SDD) range. SDD was taken as sensitive. For Nystatin 11(85%) out of 13 isolates 11 were sensitive, among them 2 were SDD and 2 were resistant.

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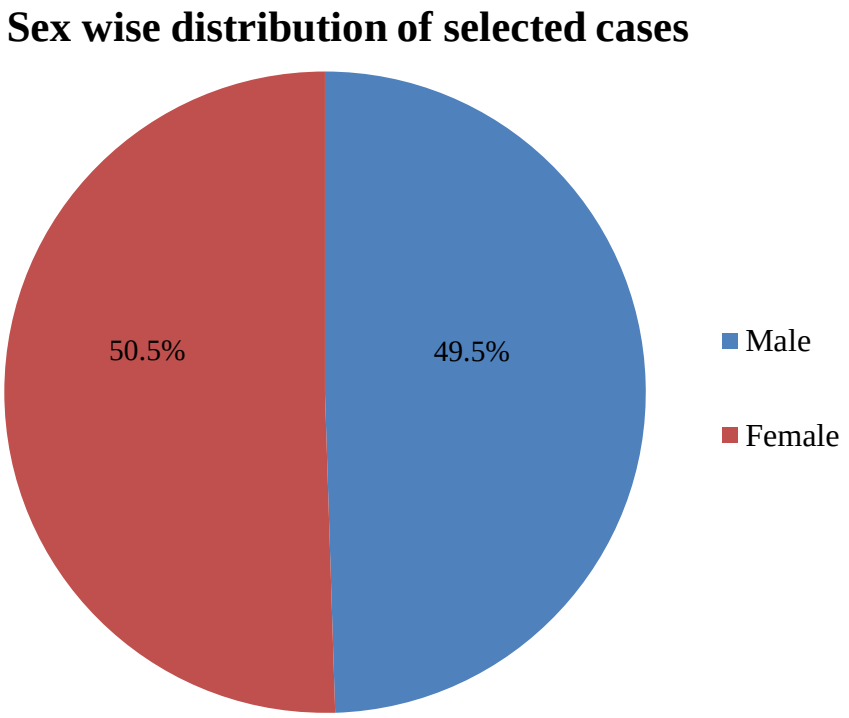

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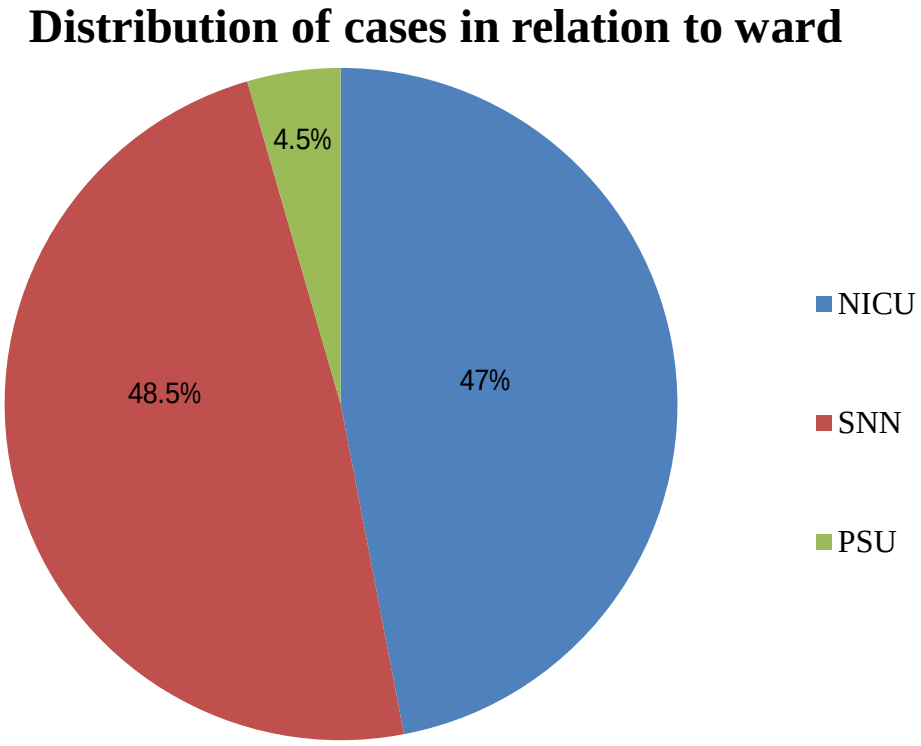

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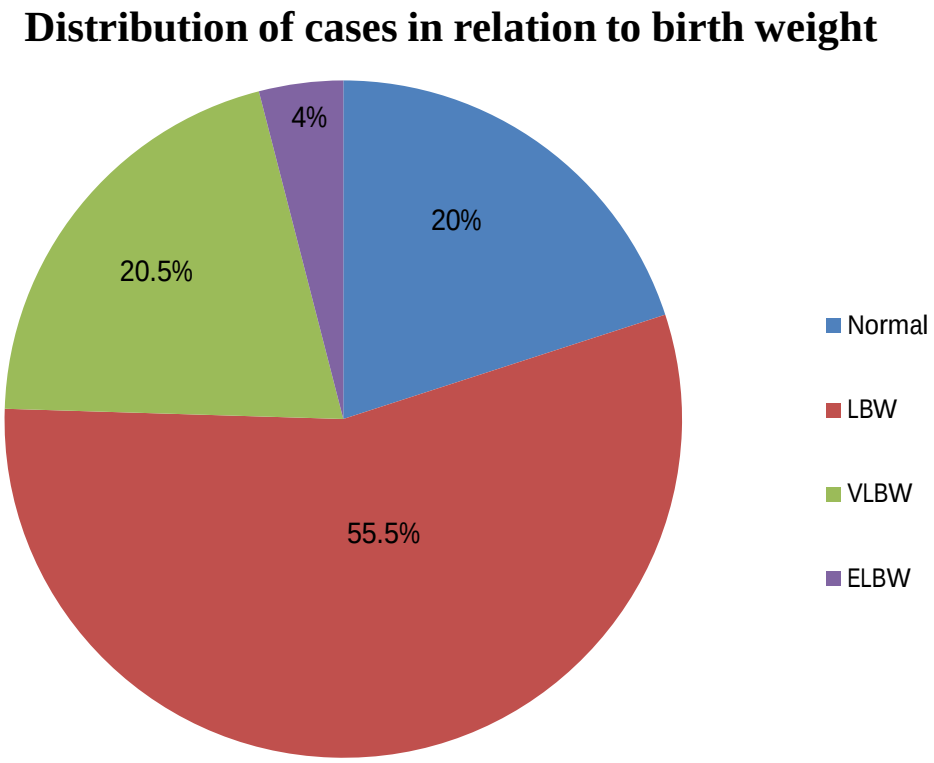

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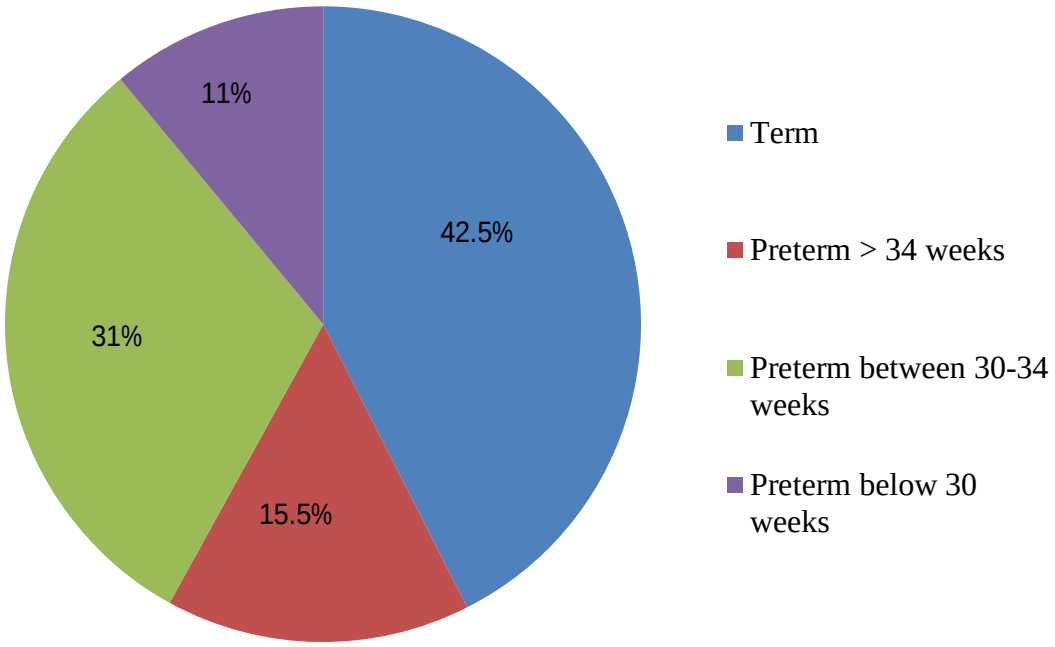

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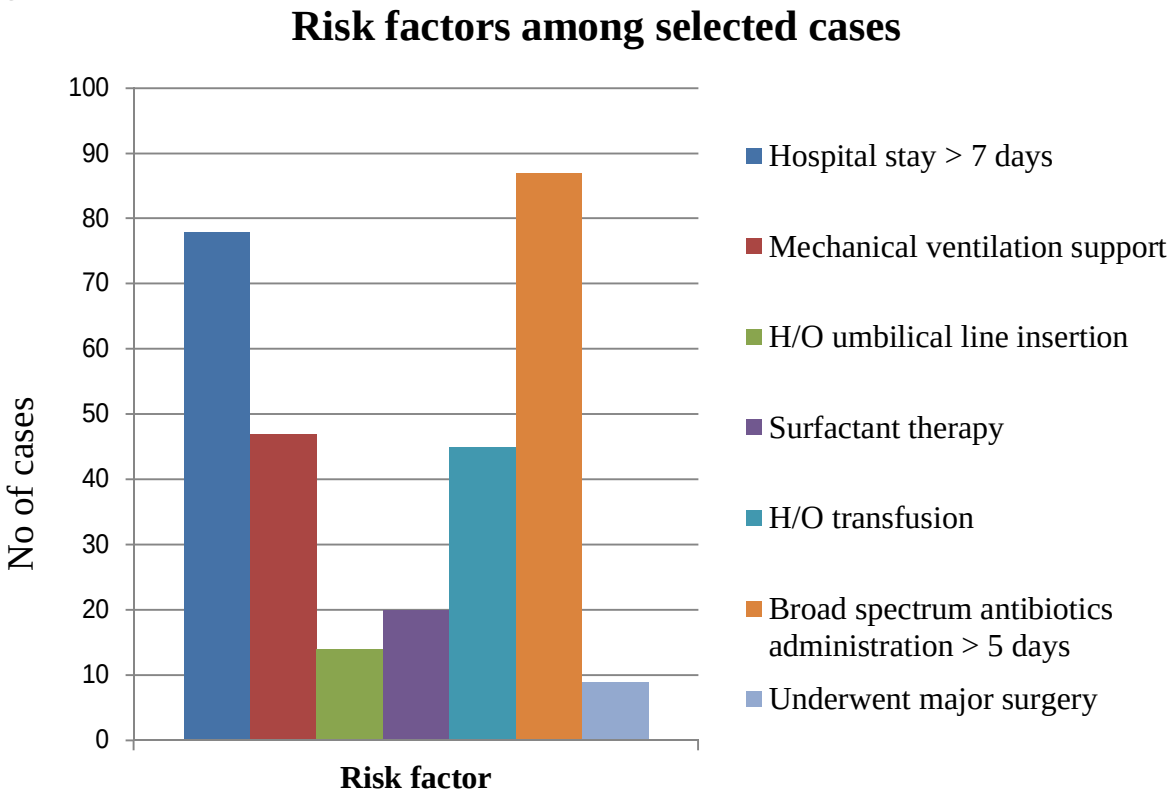

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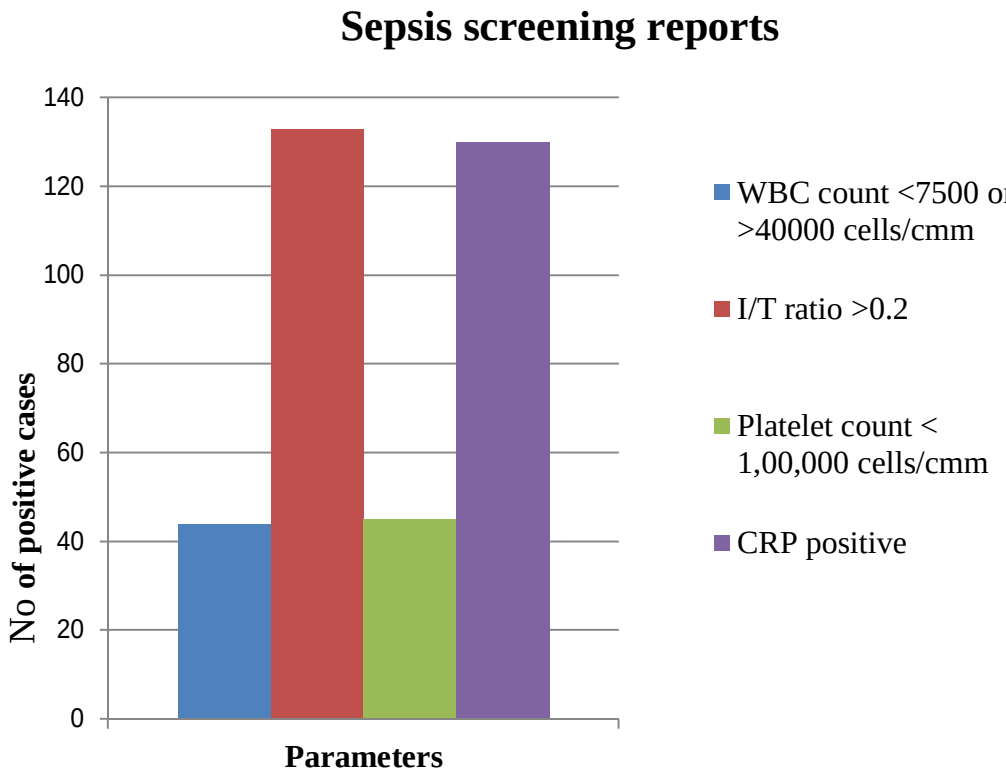

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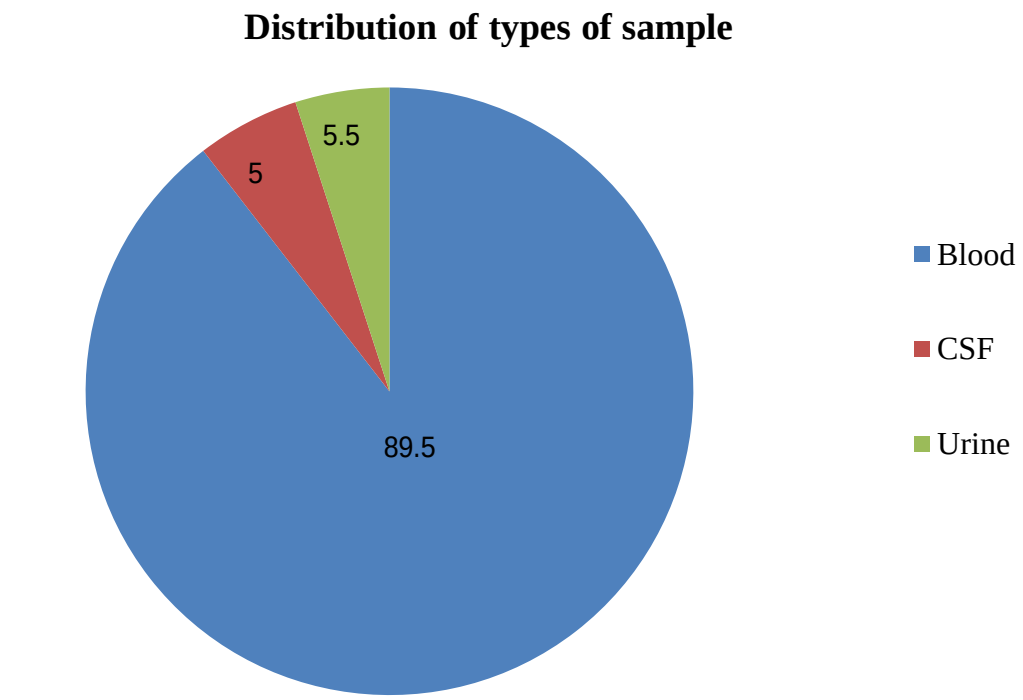

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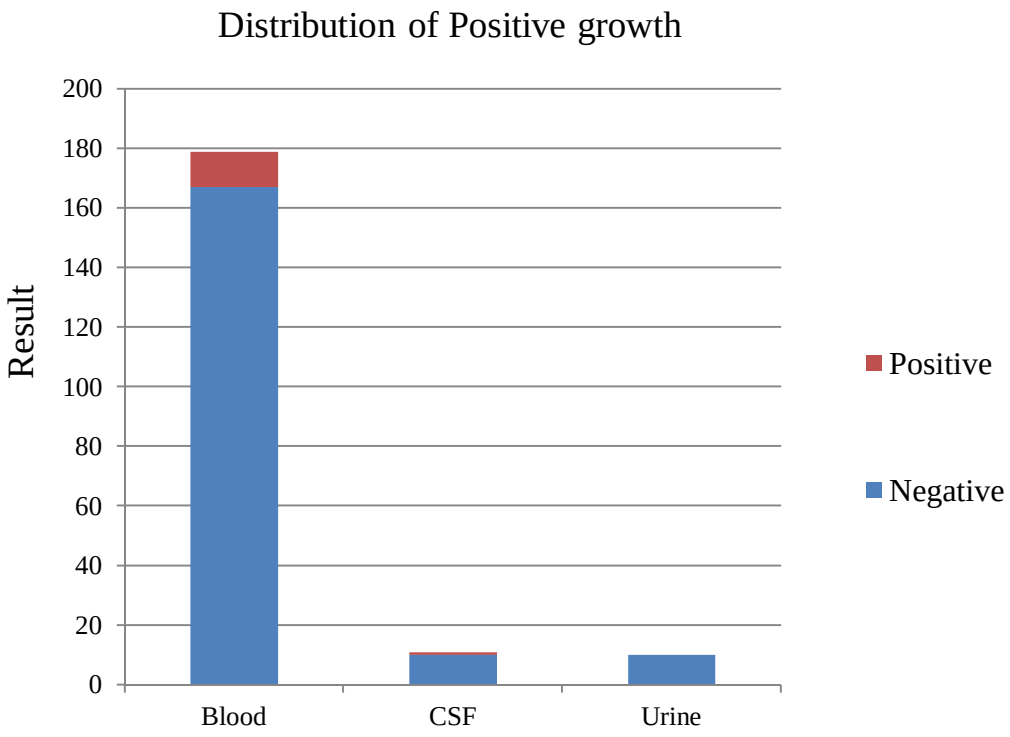

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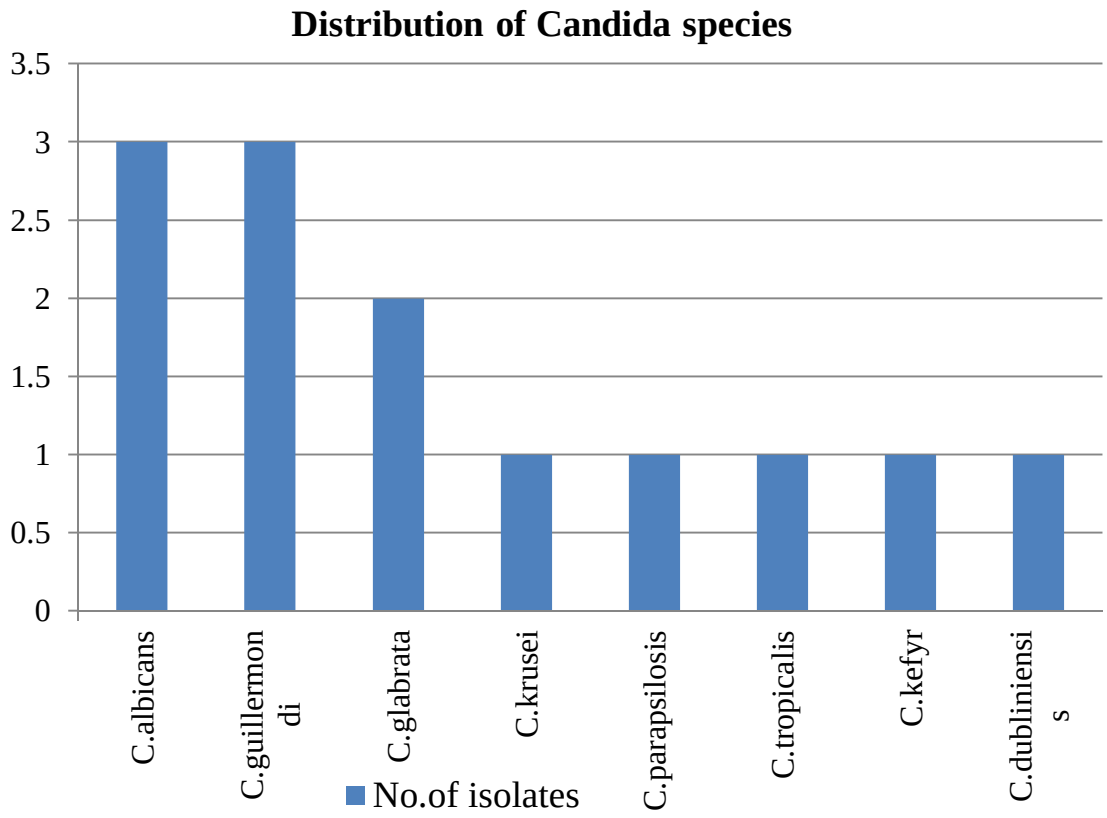

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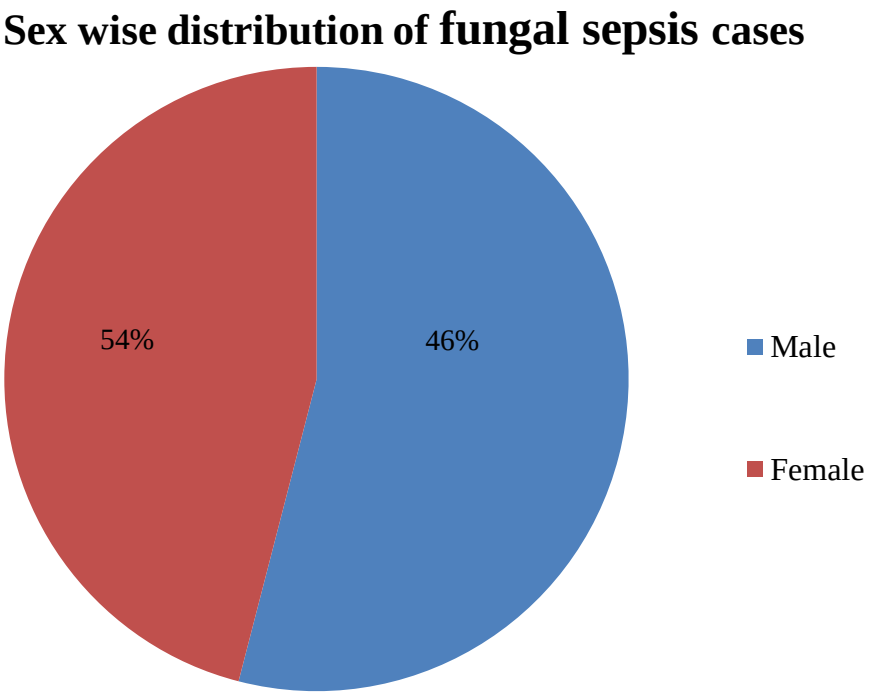


Figure 11:

Distribution of positive cases in relation to place of birth

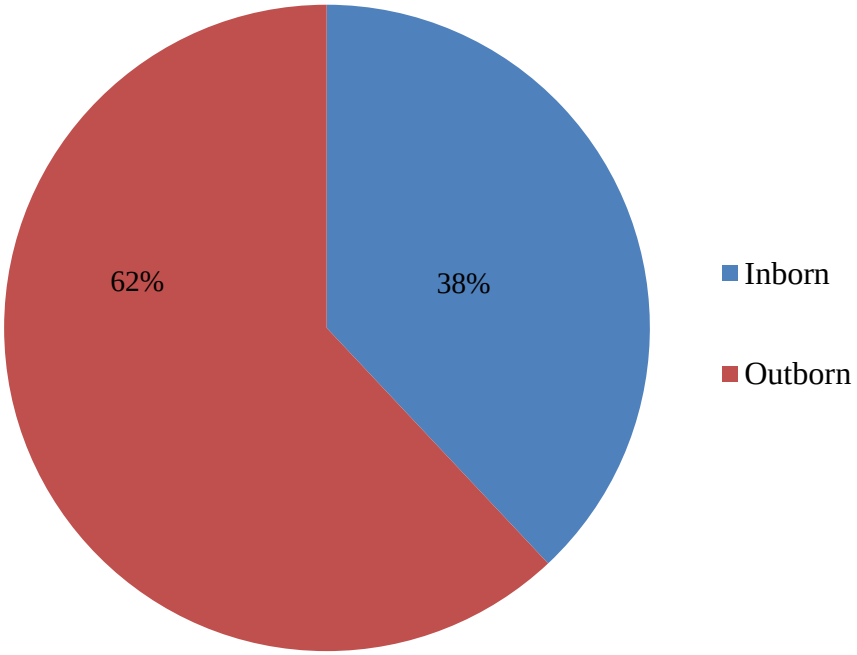


Figure 12:

Distribution of positive cases in relation to birth weight

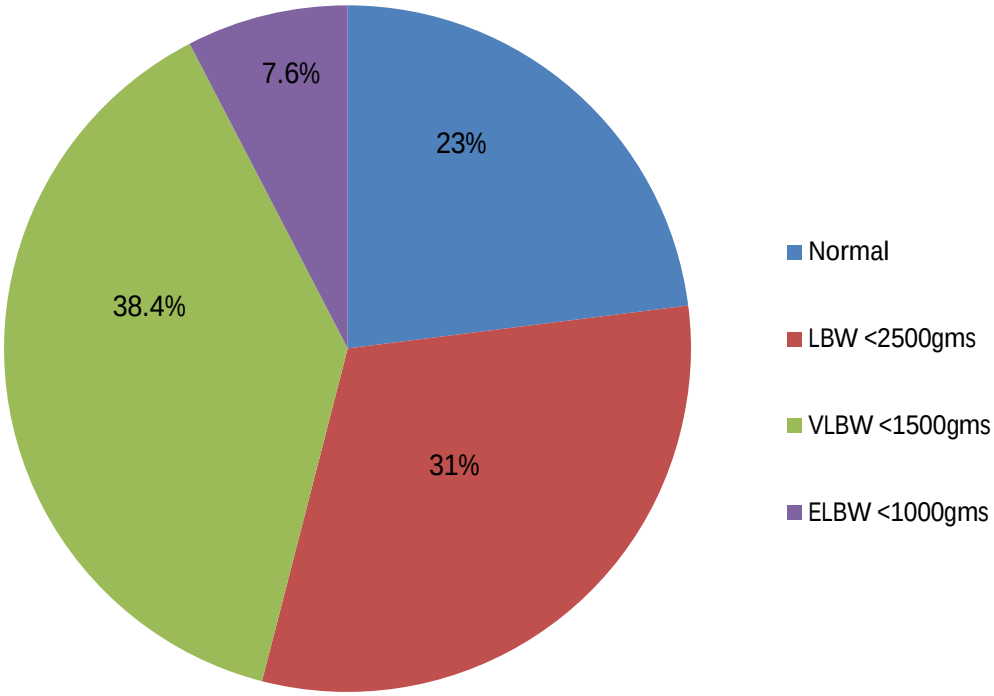


Figure 13:

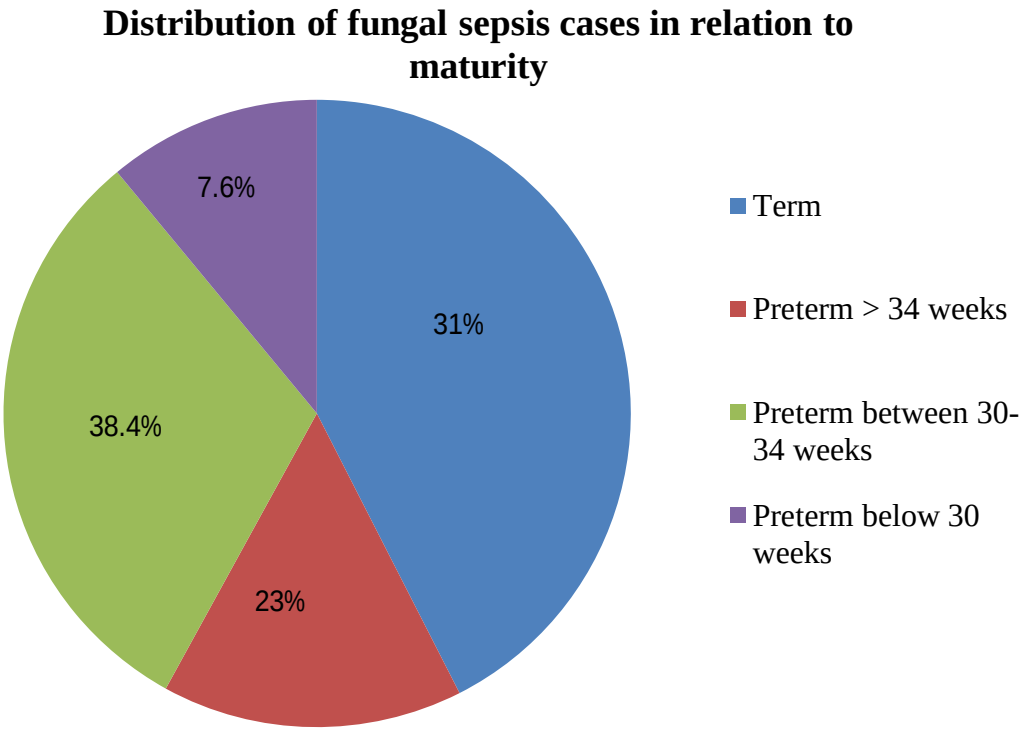


Figure 14:

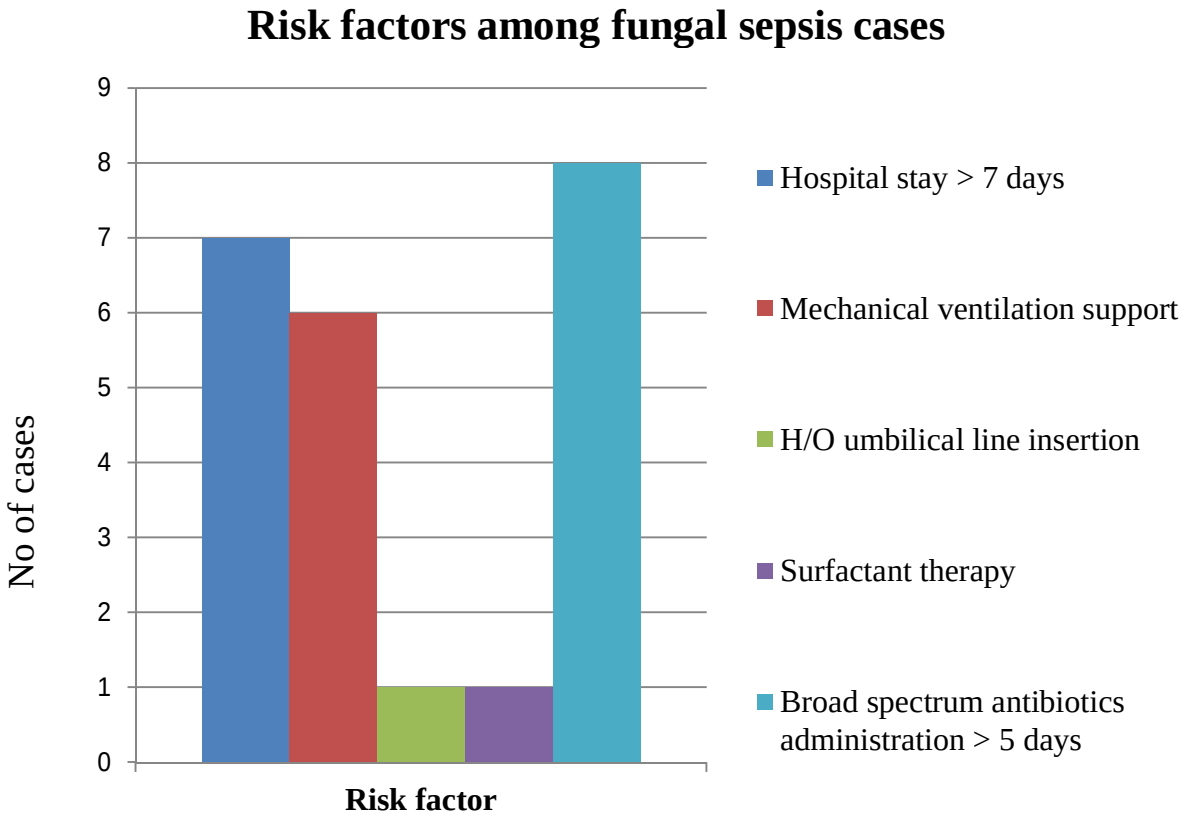


Figure 15:

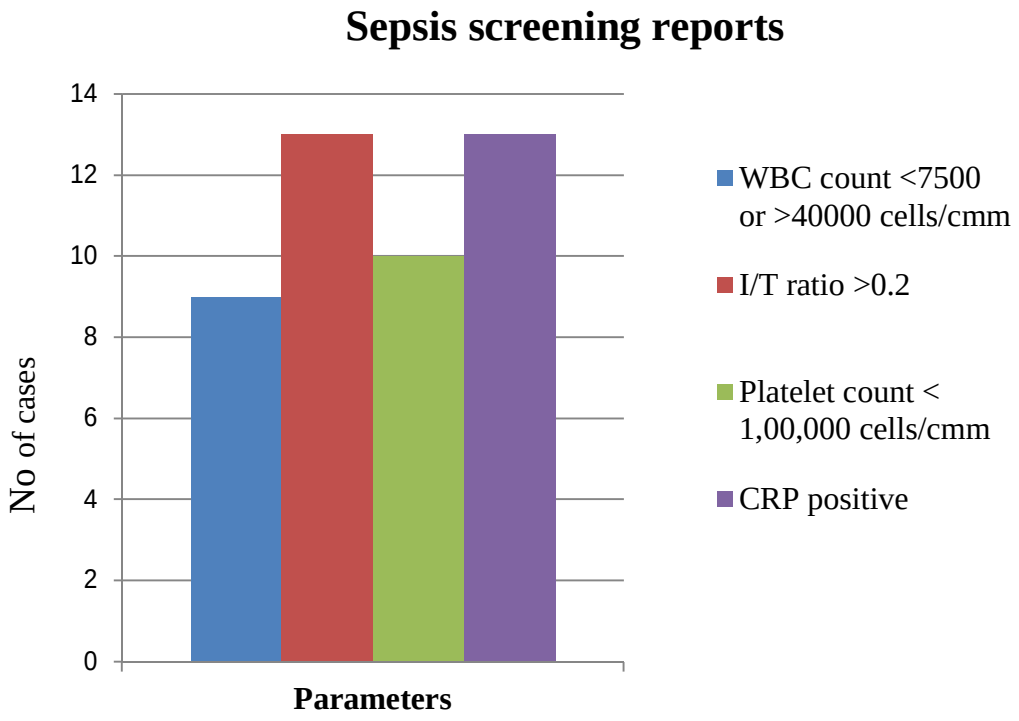
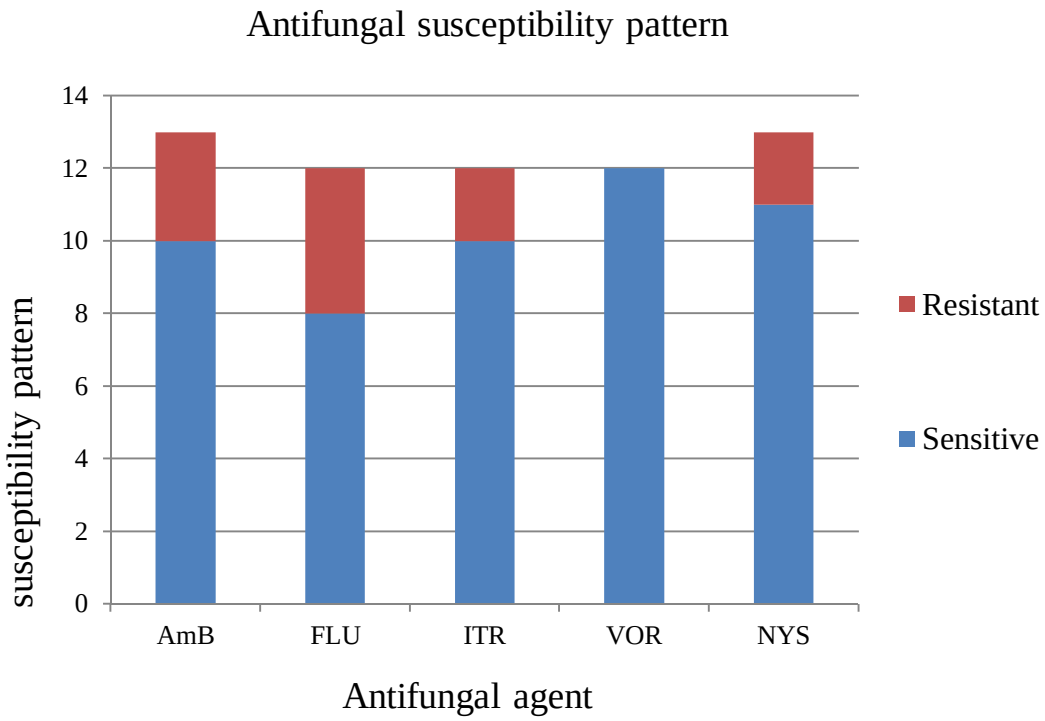


Figure 16:




DISCUSSION

Discussion

Neonatal sepsis is one of the leading causes of neonatal mortality and morbidity. Now a days, fungal pathogens are emerging as important causative agents. The severity of fungal sepsis is more than any other infections. The antifungal drug resistance is also being reported. Most vulnerable group for fungal sepsis is neonates, because of their immature immune system and this is one of the cause for increasing neonatal mortality rate. And so, there is a need for evaluation of fungal sepsis in neonates. This study is designed to carry out such evaluation in neonates.

In this study the incidence of fungal sepsis in neonates was 6.5%. This is in accordance with the study done by **Shabina Ariff et al** (2011) at Karachi, Pakistan, [123] there the incidence was in a range from 1.4% to 5.9%. This is also comparable with studies from developed countries. An Australasian study by **Howell A et al** (2009) reported the incidence of 1.6% to 5%.[50] **Paolo Manzoni et al** (2006) reported 8.1% of neonatal fungal sepsis in their study.[94] **Chapman RL, Roger GF** (2003) had observed the incidence of 2.2% to 16.5% .[19] **Stoll BJ et al** (2002) also reported the above range of incidence.[131]

Distribution of fungal sepsis cases in relation to place of birth was analyzed in this study and it was found 8 cases (62%) were among out born babies who

were delivered elsewhere and referred to the higher institutions for further management. These cases were usually admitted in Sick Newborn Nursery (SNN).This was reported in same manner by **Shabina Ariff et al** (2011), in their study there was 64% of fungal sepsis cases of out born. [123] According to the report of National Neonatal Perinatal Database and **Sankar et al(**2008) sepsis is 12 times common among out born babies admitted in tertiary care hospital.[118] In developing countries most of the deliveries are conducted at primary and secondary care hospitals, private hospitals and at home with the help of traditional birth attendants and most of them are referred to tertiary care hospital. This may be probable reason for exposure to environmental pathogens which leads to higher incidence of neonatal sepsis with invasive fungal agents.

In this study, the fungal sepsis was distributed more or less equally among both sexes and there was 7 cases(54%) in male neonates and 6 cases(46%) in female neonates.. This is in accordance with the study done by **Daynia E. Ballot et al** (2013) at South Africa, there was also male preponderance (57%).[27] This is also similar to another study done by **Paolo Manzoni** et al (2006) who reported male preponderance (54.5%).[94] The gene located at X chromosome involves with the function of thymus or with the synthesis of immunoglobulin and presence of double X chromosome in female gender leads a greater resistance to infection. This

was explained by **Bellani AJ, Schlegel** (1969), in the textbook of Infectious Disease of the Fetus and Newborn.[14]

Birth weight is one of the important criteria for selection of cases. In this study 3 cases (23%) of fungal sepsis were of normal birth weight but 10 cases (77%) were LBW neonates. The incidence is more than threefold in LBW neonates. Among this 10 cases, 6 (60%) cases are in VLBW and ELBW babies. This is similar to the study done by **Carey AJ et al** (2008), they reported a range of 44% to 75% of fungal sepsis among VLBW and ELBW babies.[18] In US, Neonatal Research Network Study (2008) observed a cumulative increase of incidence from 2.4% to 20.4% between normal and LBW neonates. Birth weight of a newborn is not only depending upon the gestational age at birth, but it also depends on maternal factors like antenatal care, nutrition, maternal infection or illness, multiple gestations, twin pregnancy, uterus and placental anomalies and fetal factors like congenital infection or anomalies and inborn error of metabolism.

Gestational age at birth decides the maturity of the newborn and indicate the immune status of each individual at birth. Gestational age at birth is inversely related to occurrence of neonatal sepsis. In this study, 9cases (69%) of fungal sepsis are preterm (below 37 weeks) and among them 5 cases are below 34 weeks of gestation, one is below 30 weeks with ELBW. This finding is in accordance with the study done by **Fridkin SK et al** (2006) at US, with clear

association in preterm birth with increasing incidence of fungal sepsis from10% to 16% for decreasing gestational age at birth.[37]

Risk factor analysis done for all of the positive cases and their association with infection and sepsis were studied. Prolonged hospital stay implicated for 54% of cases. This is similar to **Huang et al** (1998) study, there was more than 50% of sepsis among prolonged stay at hospital.[51] An Indian study by **Singh K et al,** (1999) reported 22.8% of invasive fungal infection in preterm neonates staying more than 1 week in NICU.[123] This is mainly due to cross infection in the hospital environment but it can be prevented by strict adherence to infection control practices and proper instructions to the health care workers in NICU to follow the universal precautions.

Mechanical ventilation is one of the life saving measure in severe birth asphyxia, preterm neonates with severe respiratory distress syndrome (RDS), meconium aspiration syndrome with respiratory failure, severe sepsis with multi organ dysfunction, metabolic acidosis and some cases of neonates under post poerative care. Such a important intervention may increase the chances of invasivse infection and sepsis. In this study there are 6 cases (46%) of fungal sepsis put on to mechanical ventilatiory care for various indications. This is in accordance with a study done in Taiwan by **Bai-Horang Su, Hsin- Yang Hsieh et al** (2006), and it was reported increasing incidence up to 20% in mechaical ventilation care.[10]

Umbilical line device is used as a port for giving infusions, drugs and transfusion. Umbilical catheterization should not routinely be done for all neonates and it is useful in some therapeutic interventions like exchange blood transfusion and at the time of difficulty in getting peripheral venous line. In this study, only 13 cases were on umbilical line and all of them were only for exchange blood transfusion, of these only one case (7.6%) was affected by fungal sepsis.. Prolonged duration of umbilical line may lead to more number of infections as stated by **Daniel K Benjamin et al** (2010) in their observation and a rise of incidence up to 28% was reported.[25]

Surfactant deficiency is commonly encountered in preterm babies born before 30 weeks of gestation. Surfactant therapy is given to such neonates in all tertiary care NICUs. It is administrated directly into lungs trough endotracheal tube and put on to mechanical ventilation for few hours to few days. In this study, surfactant therapy is noted as a risk factor in one case (7.6%) of fungal sepsis. **Paolo Manzoni et al** (2006) reported the association was 78% for progression to invasive fungal infection.[94] In developing countries, Surfactant therapy is given only in well established NICU and the number of such therapy are less in comparison with developed countries.

Association of broad spectrum antibiotics administration above 5 days was 64%amoung the fungal sepsis cases. This is in accordance with a study conducted

by **Rodrignez D et al** (2006) and there was more than 50% association with broad spectrum antibiotic usage**.**[110] Broad spectrum antibiotics usage may lead to suppression of normal bacterial flora of oral and gastrointestinal mucosa, it allows the overgrowth of fungal pathogen like Candida species, which progress to invasive infection. Prolonged antibiotic therapy needs a central or peripheral venous line, if it kept for prolong period it may increase the chances of colonization and invasion by fungal pathogens. So frequent changing of venous line will reduce the risk.

Most of the cases of fungal sepsis occurred as late onset sepsis .In this study about 75% of cases were in this category. This is similar to the study done by **Tushar B,Parikh et al** (2006) in India that the percentage of fungal infection detected beyond 7 days was 60% and beyond 3'rd & 4'th week was 20%.[134] In EOS, the fungal sepsis is less common and it is mostly transmitted through birth canal of the mother during delivery. The nosocomial environment is associated with more LOS cases in NICU. This is modified by well designed NICU with improved care and proper screening of suspected neonates.

There are several markers used for screening of a suspected neonatal sepsis. Among them thrombocytopenia is important indicater in fungal sepsis. CRP is elevated in most of the acute illnesses and infections. I/T ratio is also one of the valuable marker of sepsis. In this study, septic screening reports were

analyzed and compared for all fungal sepsis cases. There was 100% positivity of CRP (>1.0mg/dl), elevated I/T ratio >0.2 in all cases .Thrombocytopenia was noticed in 10 cases (76%) and decreased total count noted in 9 cases (69%).This is in accordance to the reference value charts formulated by **Monroe BL, et al** (1979) and **Mouzinho A, et al** (1994), which concludes that if there were two abnormal parameters in a screening, is 93-100% sensitivity and 83% specificity.[87,90] It was also updated by **David Kaufman** (2014), the association of thrombocytopenia with neonatal Candida infection.[26]

All of the thirteen fungal pathogens were Candida species. This is in accordance with **David A Kaufman** (2014) and **Hasen Tezer et al**(2012). They reported Candida species was the leading fungal pathogen for neonatal fungal sepsis.[26,48]

Aspergillus species infection is common among preterm neonates. It is transmitted by air born fungal spores. Infection is usually due to contaminated dust exposure. This study is conducted in a well established NICU at tertiary care centre. A clean dust free environment is maintained at all time inside the NICU. Transmission through skin is rarely encountered but it needs a primary focus for spread. So, no such fungi was isolated from the samples.

Malassezia furfur is a lipophilic fungus, which usually needed a media with lipid content like lipid infusion prepared for total parentral nutrition (TPN). TPN

is infused to most of the VLBW, ELBW, necrotizing enterocolitis(NEC) and post operative neonates. Because of the higher cost of such treatment, such therapy was not administered in the study centre, and so it was not reported.

Zygomycetes is another emerging fungal pathogen isolated in few cases of neonatal sepsis. The mode of transmission is trough skin with local trauma and intravenous line contamination. But these fungal patogens are found only in the polluted environment. In a well maintained NICU, the infection by this pathogen is less likely encountered.

Species level distribution of the Candida is analyzed in this study. There is equal distribution C. albicans(23%) and C.gullermondii (23%)among the isolates. The C. glabrata(15%) is next to these and one isolate of C. tropicalis, C. parapsilosis,C. dubliniensis, C.krusei and C. kefyr(7.6%)each**. K. Sesu kumari** et al(2014) isolated predominant non albican Candida species (65%) from various clinical samples in their South Indian study.[61] **Fridkin et al** (2006) reported a similar pattern of distribution with C. glabrata C parapsilosis, C. tropicalis and C. krusei in their study.[37] **Ali Zarei et al** (2013) isolated 48% of non albican Candida species like C. glabrata, C. tropicalis and C. krusei in candiduria study. In last decade, several reports stated that non albicans Candida is an emerging pathogen.[3] **Selma Amaral Lopes and Andressa Moura** (2012) reported three

cases of C,Krusei in their neonatal fungal sepsis study at Brazil.[122]

V P Baradkar et al(2008) also reported a case of C. dubliniensis. The less common species like C.gullermondii and C.kefyr were also isolated in this study.[135] The C.gullermondii was isolated in NICU by **K. Sesu kumari** et al(2014)) in their study.[61] It is considered as environmental pathogen and transmitted through heparin solution used to flush the needles. C.kefyr was previously designated as C. pseudotropicalis rarely involved in human infections.

Antifungal susceptibility pattern is analyzed in this study. There resistance pattern noted was as follows- Flucanazole (30%), Amphotericin B(23%) and Itraconazole(17%). Upto 15% resistances for Nystatin. All isolates were susceptible to Voriconazole . Azole drugs are not tested against C. krusei because of its intrinsic resistance. Some strains of C. krusei also have intrinsic resistances for Amphotericin B. All isolates of C. albicans, C. glabrata and C.tropicalis were resistant to more than one drug. Many stains of C. glabrata are susceptible dose dependant or intrinsically resistant to Azole drugs.

Acquired drug resistances to Azole drugs is increasingly noted in most of C. albicans isolates. **Yang YI et al** (2003) study is comparable with this study, which reported 20% to 33% of drug resistance to Azole drugs. There was 12% resistance for Amphotericin B and 48% resistance to Flucanazole among C.albicans. C. glabrata showed 4% resistance, 60% susceptible dose dependance for

Amphotericin B and 28% resistance, 64% susceptible dose dependance for Flucanazole. [146]

Nystatin is used orally to reduce gut colonisation. Amphotericin B and Flucanazole are preferred for treatment in most of the neonatal fungal sepsis. Because of the toxicity of Amphotericin B deoxycholate and high cost of liposomal preparation, fluconazole is used in many centers.

Fluconazole prophylaxis is suggested for preterm < 30 weeks of gestational age, VLBW,ELBW and acute onset thrombocytopenia neonates by **Tushar B,Parikh et al**(2006).[114] The usual regimen is 6mg/ kg for every 3rd day in the first week of life and then every 2nd day from second week up to 30 days for VLBW and 45 days for ELBW neonates.

Biochemical reactions like sugar fermentation and sugar assimilation are useful for further speciation of Candida. CHROMEagar and kit based sugar assimilation are rapid methods for identification of candida species but the cost of each test limits their routine use. Antifungal susceptibility testing must be done for all isolates to give proper guidelines for treatment and to control the emergence of drug resistance.

SUMMARY

SUMMARY

The study on Characterization and speciation of fungal isolates in neonatal sepsis in a tertiary care hospital revealed a incidence of 6.5% fungal sepsis. The important risk factors noted were male preponderance (54%), prematurity (69%), and low birth weight (77%). Most of the sepsis cases were presented as late onset sepsis (75%). Modifiable risk factors like administration of broad spectrum antibiotics (62%), prolonged hospital stay (54%) and mechanical ventilation care (46%) have played an important causative role for colonization and infection. Blood transfusion, surfactant therapy and umbilical catheterization also were associated risk factors of fungal sepsis. Thrombocytopenia was one of the important marker for fungal sepsis along with increased I/T ratio, raised CRP and reduced total WBC count. Only Candida species were isolated from the culture positive specimens. Among them C.albicans(23%) and C.gullermondii(23%) were the common pathogens. Antifungal susceptibility pattern showed 100% susceptibility to Voriconazole and 30% resistance for Fluconazole and 23% to Amphotericin B.

Presumptive identification of fungal isolates was done by wet mount, Gram staining, India ink and it was confirmed by conventional fungal culture method. Further processing of growth with microscopic methods like germ tube test and corn meal agar were done for the speciation of the organism. Sugar fermentation

and sugar assimilation tests were performed for the confirmation of osolates. Rapid kit based identification test and CHROMEagar growth were the additional tests that improved the outcome. Antifungal susceptibility pattern of the isolates were tested by disc diffusion, agar dilution methods and the MIC of the fungal isolates were determined by macro broth dilution and micro broth dilution technique, micro dilution method which is the standardized method recommended by CLSI guidelines.

CONCLUSION

CONCLUSION

The study on Characterization and speciation of fungal isolates in neonatal sepsis in a tertiary care hospital revealed the following findings:

1) Among the 200 selected cases of neonates ,fungal pathogens were isolated from 13 cases and the incidence of fungal sepsis was 6.5%

2)Maximum cases were among out born neonates(62%) those who delivered at varies centers like PHC, Government HQ hospitals, Private hospitals and even at home. There were more chances for exposure to pathogens in outside delivery, while handling and transportation to the tertiary care hospital.

3)Among the fungal sepsis cases, 54% were male neonates as female babies have a better resistance to infection by possessing double X chromosome which are the dominant factor in immune development.

4) Prematurity (69%) and low birth weight (77%) were major risk factors for fungal sepsis. Immature immune system among them was the main factor of concern.

5) Among the risk factors, administration of broad spectrum antibiotics (62%), prolonged hospital stay for more than 7 days (54%) and transfusion (54%) had major contributions. Intervention like mechanical ventilator care (46%), umbilical catheter insertion (7.6%) and surfactant therapy (7.6%) were also implicated as important risk factors. The causative role of the above factors is

modifiable one by creating awareness and giving better training for all health care workers.

6) Thrombocytopenia (platelet count <1,00,000 cells/ cmm), elevated I/T ratio > 0.2 and raised CRP were the relevant septic screen markers. Presence of these markers in neonates with suspected sepsis will gives clue to rule out fungal sepsis.

7) Conventional culture methods by itself is adequate to identify and speciate the fungal pathogen.

8) Candida species were the only pathogen isolated from samples. C.albicans (3), C. guillermandi (3) & C.glabrata (2), each one of C.tropicalis , C.parapsilosis, C.kefyr, C.krusei, C.dubliniensis were isolated from the clinical samples.

9) Resistant pattern observed for Fluconazole (30%), Amphotericin B (23%), followed by Itraconazole (17%), Nystatin (15%). All isolates were susceptible to Voriconazole. Voriconazole is used as the second line drug for most of the resistant fungi now a days. Antifungal susceptibility testing and MIC determination by microbroth dilution method will guide in selecting appropriate antifungal agents and to reduce the emergence of resistance.

10) Proper care for preterm neonates, restricted usage of broad spectrum antibiotics, early removal or replacement of indwelling catheters or devices,

reducing the unnecessary intervention, early initiation of enteral feeding and earlier discharge will help in controlling the nosocomial spread of fungal pathogen.

11) Prevention of fungal sepsis starts from antenatal period. Proper screening for genital infection and treatment during ANC period is the first step. Standard precaution should be followed in every hospital for a clean delivery. Transit of sick neonates with all precaution will help to avoid exposure to pathogens. Avoidance of unwanted admissions, early discharge of healthy neonates and following universal precaution will reduce the disease burden.

12) As neonatal sepsis is one of the major cause for neonatal mortality and morbidity, evaluation of suspected cases for bacterial and fungal pathogens are immense important. Similar studies about screening and surveillance of fungal pathogen in neonatal sepsis will throw light on the scenario of fungal sepsis.

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ANNEXURE

Annexure -i

REAGENTS

1.Gram staining reagents;

1.Primary dye - Methyl Violet - 0.5%

2.Gram's iodine solution

3. Decolorizer - Acetone.

4. counter stain - dilute carbol fuchsin (1:10)

Gram's iodine prepared by dissolving 2 g of potassium iodide in 25 ml of water, then adding 1 g of iodine crystal and make up to 100 ml with distilled water.

2.LACTOPHENOL COTTON BLUE STAIN (LPCB)

Useful as a fluid mount for microscopic observation of fungi.

Phenol	20 gm [Melt in warm water then weigh]
Lactic acid	20 gm
Glycerol	40 gm
Cotton blue	0.05 gm
D. W.	20 ml

Add - lactic acid, Glycerol, Phenol and DW

Then dissolve with even heat

Annexure -ii

MEDIA

1. SABOURAUD DEXTROSE AGAR (SDA)

Emmons modification

Dextrose	20 gm
Neopeptone	18 gm
Agar	20 gm
Distilled water	1000 ml
Final pH	6.8 - 7

Dissolve the ingredients by boiling, dispense in tubes and autoclave at 121°C for 15 mins.

Allow the tubes to cool in slanted position.

Store in refrigerator at 4°C.

Shelf life of 30 days in test tubes and 14 days in petri dishes.

SDA with gentamicin and chloramphenicol

Gentamicin	0.5 mg/ml
Chloramphenicol	0.05 mg/ml

Add 50 mg of Gentamicin in 10 ml of acetone and 50 mg of Chloranphenicol in 10ml 95% alcohol and add these to the boiling Sabouraud medium.

Mix the medium thoroughly. Tube and autoclave at 121℃ 10 min.

2. POTATO DEXTROSE AGAR (PDA)

(Peel, slice, and boil potatoes in 100 ml of distilled water for 1 hour filter and add the following)

Dextrose	20.0 gm
Agar	20.0 gm
Distilled water	To make final volume to 1 lt.

Bring to boil to dissolve agar.

Autoclave at 121℃ for 15 minutes.

3.CORNMEAL AGAR (CMA)

Cornmeal Agar with Tween 80

Yellow cornmeal	40 gm
Distilled water	1000 ml
Agar	20 g

Heat Cornmeal in a water bath in 60℃ for 1hr.

Filter through gauze.

Bring the level of filtrate to 1000 ml.

Add 2% Agar.

Autoclave at 121℃ for 15 minutes.

To plain cornmeal Agar add Tween 80(10 ml)

and pour in sterile petri dishes.

Shelf life is 14days.

3.Christensen's urea agar

Glucose	5 g
Sodium chloride	5 g
Potassium dihydrogen phosphate	2 g
Peptone	1 g
Agar	20 g
Water	1 lit

4. Brain Heart Infusion (BHI) broth

This is reconstituted from dehydrated form (brought from Hi Media Lab, Mumbai) according to manufacturer's instructions.

Miniature bottles (15 ml) were used and 5 ml of BHI broth was filled in each bottles

Autoclaved with caps tight at 121℃ for 15 mts.

Used in neonates where the small volume of blood collection (0.5-1ml) was advised.

5. CARBOHYDRATE FERMENTATION MEDIA FOR YEASTS (sugars used are dextrose, maltose, sucrose, lactose, galactose, trehalose & cellobiose)

Sugar	1.0 gm
Nutrient broth	100 ml
Bromothymol blue (0.2% alcoholic)	1.2 ml

distribute as 5 ml per tube and introduce Durham's tube inside all broth, autoclave at 115°C for 10 minutes.

6. CARBOHYDRATE MEDIA FOR ASSIMILATION OF YEASTS

a) Yeast nitrogen base agar

Yeast nitrogen base	0.67 gm
Agar	20 gm
Distilled water	1000 ml

autoclave at 115°C for 15 minutes prior to use.

Carbohydrate solution (20%) (sugars used are dextrose, maltose, sucrose, lactose, galactose, melibiose, trehalose, cellobiose, inositol, xylose, raffinose, & dulcitol)

Sugar	20 gm
Distilled water	100 ml

Autoclave at 115°C for 10 minutes and store as stock solution.

7. Mueller- Hinton agar (with+ 2% dextrose and 0.5 µg of methylene blue dye/ml)

Beef infusion	300 ml
Casein hydrolysate	17.5 g
Starch	1.5 g
Agar	10 g
Distilled water	1 lit

Dissolve the constituents by gentle heating at 100°C with agitation

Sterilize by autoclaving at121°C for 15 mts.

Add sterile methylene blue dye 0.5 µg/ml and pour in sterile petri dishes

8. Micro broth Dilution antifungal susceptibility Testing

Specimen Requirements

Yeast

All yeast isolates must be streaked for isolation onto Potato Dextrose agar (PDA). Bacterial contamination interferes with testing.

Subculture yeast to PDA agar quadrants and incubate at 30°c for 24-72 hours prior to testing.

Reagents

Antifungal Drugs

RPMI-1640

Sterile Distilled Water

Dimethyl sulfoxide (DMSO)

Sabourarud Dextrose Agar

Equipments

Spectrophotometer /Densicheck

Incubator

Plate reader

Multi-channel pipette

Adjustable pipette in 1-1000 ranges.

Supplies

0.22 nm filter system

10 ml pipette

5 ml pipette

15 ml centrifuge tubes

12*75 snap cap tubes

Sterile wooden applicator sticks

96-well round bottom microtiter

Pipette tips

Sterile transfer pipettes

Media Preparation

Broth medium was RPMI 1640 broth buffered with MOPS buffer (0.165 M) and 0.2% dextrose and glutamine without sodium bicorbanate to a pH of 7.0. Media was brought from HiMedia laboratories Pvt. Limited, Mumbai

Concentration tested

Concentrations should reflect the breakpoint concentrations and/or the expected results for the QC strains.

0.03-16 µg/ml

Amphotericin B

0.125-64 µg/ml

Fluconazole

Drug stock preparation

Amphotericin B

Pharmaceutical grade powder-refrigerated

Using calculation, determine amount of powder to weigh for 10ml in 1600 µg/ml stock.

Dilute powder in 10 ml DMSO (100%)

Store 2.0 ml 1600 µg/ml aliquots at -70°c for one year

Makes 5 stocks ready for drug set preparation

Drug dilution sets are prepared in 100% DMSO with a final volume of 1ml of each concentration and stored at -70°c for one year

Remove sets as necessary to prepare plates.

Fluconazole: Pharmaceutical grade powder-room temperature

Using calculation, determine amount of powder to weigh for 10 ml of 6400µ g/ml

Dissolve powder in 10 ml DMSO 100%

Freeze 2 ml of 6400 µg/ml aliquots at -70°c for one year

Remove sets as necessary to prepare

Plate preparation

Remove drug set from freezer and allow to thaw

Label plates with drug initials on top side panel and concentrations under Wells along the bottom top panel

Determine the amount of RPMI required. To prepare plates by multiplying the desired number of plates by 0.8. This will provide the minimum amount of media required to make plates. Add one additional MLS to this amount to account for pipetting errors, etc.

Label media tubes with desired end concentration for tubes 1- 10. Mark tubes 11-12 with C for control.

Dispense the desired volume of RPMI in to each tube

Add the corresponding drug/diluent (for controls) to each labeled tube In a 1:50 ratio. To determine the amount to add, use the followig equation (RPMI volume in this sample is 10 ml):

1/49 = x/10

49x=10

X=0.204

Add 204 l DMSO to each of the 2 Control tubes

Add 204 l of each drug dilution to each labeled tube containing 10 ml of RPMI

Dispense 200µ l DMSO Control in column 12 and 100µl in column 11

Dispense 100µ l drug to appropriate columns using a multi-channel pipette

Inoculum preparation yeast

Yeast 0.5-2.5 * 10^{5}

Subculture yeast isolates on PDA for 24-72 hours prior to setup and incubate at 30°c

Remove 24-72 hour subcultures from 30°c incubator.

Label 12 * 75 snap cap tube with FTL# and add approximately 3.0 ml sterile distilled water to the tube

Place tube within the spectrophotometer/densicheck and adjust to 0 OD

Using a sterile wooden applicator stick add a small amount of appropriate yeast isolate to the tube

Measure the OD and adjust to target of 90%T (Spectrophotometer) or 0.45-0.55 OD densicheck

If %T/OD is 92 or higher/0.44 or lower, add more yeast. If 88/0.55 or higher, Add more water. Continue until target %T/Odd is reached.

Place yeast suspension in refrigerator or ice chest until ready for use.

Yeast suspensions should be used within 2 hours of preparation.

Inoculating Plates

Yeast

Add 10µl to each 5 ml of media

Vortex and pour media into sterile Petrified dish

Add 100 µl of inoculum to each well (1-11) in appropriate row

Incubation

Place plates in a plastic bin with a water reservoir

Place bin in 35°c incubator

For isolates that do not grow at 35°c within 72 hours, place plates at room temperature until growth is detected.

Reading results yeast

Time of reading for Amphotericin B, 24 or 48 hours and for fluconazole 48 hours.

MIC by visual examination measured for Amphotericin B as lowest drug concentration that prevents any discernible growth (100% inhibition).

MIC by visual examination measured for Fluconazole as lowest drug concentration that shows prominent (~50%) decrease in turbidity.

9. Macro broth dilution method

This was done in test tubes instead of microtiter wells used in micro broth dilution method.

Test inoculum preparation with a dilution of 1:2000made from stock inoculum suspension; inoculum size after inoculum, 0.5×10^3 to 2.5×10^3 CFU/ml.

Drug dilution was done by adding drug with additive (DMSO) 10 X dilutions for Fluconazole and drug with solvent (DMSO) 100 X dilution for Amphotericin B.

Drug and inoculum mixing done by adding 0.1 ml of drug and 0.9 ml of inoculam to small sterile test tubes. 10 tubes for different drug range ,11th tube for inoculam control and 12th one for media control .

ANNEXURE- iv

Master Chart

Key to the Master Chart:

NI - NICU, S - SNN, PS - Pediatric Surgery,

I - Inborn, O - Out born,

M - Male, F - Female,

T - Term, P - Preterm,

NW - Normal birth weight, L - LBW, V - VLBW, E - ELBW,

Y -Yes, N - No,

B - Blood, CSF - Cerebro Spinal Fluid, U - Urine,

G - Growth, NG - No Growth.

1	Serial No	1	2	3	4	5	6	7	8	9	10
2	Lab ID No	1025	1059	1055	1121	1134	1136	1122	1127	1153	1154
3	Ward (NICU/SNN/PSU)	NI	NI	NI	NI	S	NI	NI	S	S	S
4	Place of Birth(Inborn/Out born)	I	I	I	I	O	I	I	O	O	O
5	Age in days	8	7	8	3	6	5	5	25	6	10
6	Sex(Male/Female)	M	F	M	F	F	M	F	M	M	F
7	Maturity (preterm/term/post term)	PT	PT	PT	PT	T	PT	PT	T	PT	PT
8	Birth Weight(N/LBW/VLBW/ELBW)	L	L	L	L	L	L	V	NW	L	L
9	Use of surfactant(Yes/No)	N	N	N	N	N	N	N	N	N	N
10	Use of umbilical line(Yes/No)	N	N	N	N	N	N	N	N	N	N
11	Administration of broad spectrum antibiotics/Duration	Y/5	Y/6	Y/7	Y/3	Y/6	Y/5	Y/5	Y/7	Y/6	Y/5
12	H/O Transfusion(blood/blood products/exchange)	N	N	N	N	N	N	Y	N	N	N
13	Duration of hospital stay	8	7	8	3	5	5	5	8	6	5
14	Mechanical ventilator care (yes/No)/Duration	N	N	N	Y/3	N	N	Y/3	N	N	N
15	H/O Major surgery(Yes/No)	N	N	N	N	N	N	N	N	N	N
16	Total cell count(cells/cmm)	6000	7500	9000	5500	14000	9500	14000	3400	12800	13000
17	Immature to total neutrophil ratio(I/T ratio)	0.2	0.2	0.24	0.34	0.2	0.3	0.36	0.25	0.32	0.24
18	Platelet count(Cells/cmm)	1.5l	2.4l	1.4l	66000	-	1.2l	34000	68000	2.3l	2.5l
19	CRP(+ve/-ve)	-	-	-	+	+	+	+	+	+	+
20	Proven bacterial sepsis by culture(Yes/No)	N	N	N	N	N	Y	N	Y	Y	Y
21	Nature of specimen processed(Blood/CSF/Urine)	B	B	B	B	B	B	B	B	B	B
22	Fungal culture report(growth/no growth)	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

1	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
2	1155	1166	1182	16704	1193	1202	1225	1226	1231	1273	1250	1254	1255	1257	17620	17622	1268
3	S	NI	S	S	NI	NI	S	S	S	S	S	S	S	S	S	S	NI
4	O	I	O	O	I	I	O	O	O	O	O	O	O	O	O	O	I
5	7	8	10	5	7	11	5	12	7	3	5	13	7	3	5	6	12
6	M	M	F	M	M	F	M	M	M	F	F	F	F	M	M	M	M
7	T	T	P	T	T	P	PT	P	P	P	P	T	P	PT	P	T	T
8	L	L	L	NW	NW	L	L	V	NW	V	L	L	V	V	L	L	L
9	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	N	N	N
10	N	N	N	N	N	N	N	Y	N	N	N	N	N	N	N	N	N
11	Y/7	Y/7	Y/5	Y/3	Y/5	Y/7	Y/5	Y/5	Y/3	Y/3	Y/5	Y/7	Y/5	Y/3	Y/5	Y/5	Y/7
12	7	8	5	5	7	11	5	5	5	3	5	7	7	3	5	5	12
13	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
14	N	N	N	Y/3	N	N	N	N	N	N	N	N	Y/3	N	Y/3	N	N
15	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
16	6700	16000	12500	2800	4400	7200	8800	4200	12000	4400	9200	-	6800	-	8600	4200	12000
17	0.2	0.22	0.28	0.36	0.2	0.2	0.27	0.30	0.3	0.2	0.2	-	0.4	-	0.3	0.2	0.26
18	1.5L	68000	1.2L	60000	1.6L	3.2L	1.8L	1.1L	1.2L	98000	1.4L	-	44000	-	78000	68000	4.1L
19	-	+	+	+	-	-	+	+	+	+	-	-	+	-	+	+	+
20	N	Y	Y	Y	N	N	Y	Y	Y	Y	N	N	N	N	N	N	N
21	B	B	B	CSF	B	B	B	B	B	B	B	B	B	B	CSF	CSF	B
22	NG	NG	NG	G	NG	NG	NG	NG	NG	NG	NG	NG	G	NG	NG	NG	NG

1	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
2	1268	1272	1300	1304	1315	1319	1320	1331	1332	1352	1354	1355	1364	1380	1382	1383	1402
3	S	S	S	S	S	NI	NI	NI	S	NI	S	S	NI	NI	S	NI	NI
4	O	O	O	O	O	I	O	I	O	I	O	O	I	I	O	I	I
5	7	8	7	19	5	15	5	8	5	8	21	6	8	3	8	7	5
6	F	F	M	F	M	F	M	F	F	M	M	F	F	M	F	F	F
7	T	T	T	T	PT	T	PT	T	PT	T	T	PT	T	PT	T	PT	PT
8	NW	L	NW	NW	L	NW	V	L	L	NW	L	L	NW	V	L	L	L
9	N	N	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N
10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
11	Y/5	Y/4	Y/3	Y/7	Y/4	Y/7	Y/3	Y/7	Y/3	Y/7	Y/5	Y/5	Y/8	Y/3	Y/3	Y/7	Y/5
12	5	4	3	8	4	15	5	8	3	8	8	6	8	3	3	N	N
13	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
14	N	N	N	Y/3	N	N	Y/2	N	N	N	N	N	N	N	Y/3	N	N
15	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
16	-	14000	8800	6200	6500	8400	4400	11000	12200	14000	-	12000	10000	-	12000	9800	14400
17	-	0.3	0.23	0.24	0.2	0.25	0.3	0.32	0.2	0.33	-	0.27	0.4	-	0.2	0.4	0.34
18	-	1.6L	2.8L	2.2L	1.6L	1.8L	78000	2.6L	1.4L	2.2	-	2.6	90000	-	1.6L	1.2L	3.2L
19	-	+	+	+	-	+	+	+	+	-	-	+	+	-	+	P	P
20	N	Y	Y	Y	N	Y	Y	Y	Y	N	N	Y	Y	N	Y	Y	Y
21	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
22	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	G	NG	NG	NG	NG

1	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61
2	1403	1404	1405	1444	1447	1480	1481	1511	1512	1513	1517	1518	1520	1524	1536	1537	1538
3	NI	NI	S	S	NI	NI	NI	NI	NI	NI	NI	S	NI	S	S	S	NI
4	I	I	O	O	I	I	I	I	I	I	I	O	I	O	O	O	I
5	20	16	8	9	7	4	7	7	7	5	11	15	7	3	8	14	6
6	M	M	M	M	M	M	M	M	M	F	M	M	F	M	M	F	F
7	T	T	T	T	T	PT	T	T	PT	T	T	PT	T	PT	PT	T	PT
8	NW	NW	L	L	L	V	L	NW	L	L	NW	L	L	V	L	L	L
9	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
11	Y/7	Y/7	Y/5	Y/7	Y/5	Y/3	Y/5	Y/5	Y/3	Y/3	Y/5	Y/7	Y/5	Y/3	Y/5	Y/5	Y/6
12	N	N	N	Y	N	N	N	N	N	Y	N	N	N	Y	Y	N	N
13	20	16	5	8	7	4	7	7	7	5	11	10	7	3	Y	5	6
14	N	N	N	Y/3	N	Y/3	N	N	N	N	N	N	N	N	Y/3	N	N
15	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
16	8800	9600	12500	4000	16000	8800	16000	18000	19000	1400	7000		20000	4800	4600	14000	12000
17	0.32	0.22	0.2	0.3	0.25	0.2	0.2	0.33	0.3	0.24	0.25	-	0.4	0.38	0.3	0.32	0.26
18	2.4L	1.6L	1.2L	38000	2.6L	3.2L	1.8L	1.1L	1.2L	1.9L	1.4L	-	1.4L	96000	68000	1.8L	2.1L
19	+	+	+	+	+	-	+	+	+	+	+	-	+	-	+	-	+
20	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	N	N	N	Y
21	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
22	NG	NG	NG	G	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	G	NG	NG

1	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76
2	1548	1549	1578	1579	1582	1596	1636	1637	1646	1648	1650	1652	1665	1666	1667
3	NI	S	NI	NI	S	NI	S	S	PS	S	NI	NI	PS	NI	NI
4	I	O	I	I	I	I	O	0	I	O	I	I	O	I	I
5	7	7	8	8	32	7	4	4	7	8	8	8	4	7	5
6	F	M	F	F	F	F	M	M	M	F	M	M	M	F	M
7	T	PT	T	T	T	T	P	P	T	P	P	T	T	P	P
8	L	V	L	L	NW	L	V	E	NW	L	L	NW	NW	L	L
9	N	N	N	N	N	N	Y	Y	N	N	N	N	N	N	N
10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
11	Y/5	Y/7	Y/3	Y/3	Y/7	Y/7	Y/4	Y/4	Y/7	Y/3	Y/7	Y/7	Y/4	Y/7	Y/4
12	N	N	N	N	N	N	Y	Y	Y	Y	N	N	N	N	N
13	7	7	8	8	11	7	4	4	7	3	8	8	4	7	5
14	N	N	N	N	Y/3	N	Y/1	Y/3	N	N	N	N	N	N	N
15	N	N	N	N	N	N	N	N	Y	N	N	N	Y	N	N
16	12300	4600	22000	8100	7600	12000	18000	5600	18000	18600	8400	13000	12000	14800	8500
17	0.2	0.4	0.3	0.2	0.4	O.23	0.2	0.36	0.36	0.2	0.3	0.2	0.4	0.3	0.2
18	2.2L	60000	87000	1.1L	2.0L	1.4L	1.4L	44000	1.2L	62000	1.4L	2.1L	72000	2.6L	1.4L
19	+	-	+	+	+	+	-	+	-	+	-	+	+	+	+
20	Y	N	Y	Y	Y	Y	N	Y	N	Y	N	Y	N	Y	Y
21	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
22	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

1	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91
2	1669	1671	1672	1691	1693	1694	1696	1713	1726	1727	1728	1729	1744	1747	1748
3	S	NI	NI	NI	S	NI	NI	NI	S	NI	NI	NI	NI	S	PS
4	O	I	I	I	O	I	I	I	O	I	I	I	I	O	I
5	8	5	10	8	5	8	5	5	11	6	5	4	7	6	10
6	F	F	M	F	M	F	M	M	F	F	F	F	F	F	M
7	P	T	P	T	P	T	T	P	P	P	P	P	T	P	T
8	L	NW	V	NW	V	L	L	L	L	V	L	V	V	L	L
9	N	N	Y	N	N	N	N	N	N	N	N	Y	N	N	N
10	N	N	N	N	N	Y	N	N	N	N	N	Y	N	N	N
11	Y/3	Y/4	Y/9	Y/4	Y/4	Y/8	Y/4	Y/4	Y/7	Y/6	Y/5	Y/4	Y/7	Y/3	Y/5
12	N	Y	Y	Y	N	Y	Y	N	N	N	N	Y	Y	N	Y
13	3	5	10	8	4	8	5	5	8	6	6	4	7	6	8
14	N	Y/3	Y/4	N	N	N	Y/4	Y/3	N	Y/2	N	Y/Y	N	N	N
15	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y
16	13200	6800	4400	12300	14000	9000	5400	11000	23000	12000	6800	9700	21000	15000	16000
17	0.23	0.2	0.3	0.2	0.2	0.23	0.3	0.2	0.2	0.22	0.24	0.3	0.4	0.2	0.25
18	2.3L	67000	87000	1.2L	2.0L	1.1L	80000	2.2L	1.6L	2.2L	1.7L	96000	75000	1.9L	1.5L
19	+	+	+	-	+	+	+	+	+	-	+	-	+	+	-
20	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	N	Y	Y	N
21	B	B	U	B	B	B	B	B	B	B	B	U	B	B	B
22	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

1	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107
2	1749	1754	22712	3777	3467	1762	1763	1768	1777	1778	1779	1781	1803	1804	1808	1833
3	S	S	S	NI	S	S	NI	NI	NI	NI	NI	S	S	NI	S	S
4	O	O	I	I	O	O	I	I	I	I	I	I	O	I	O	O
5	3	8	28	7	5	7	6	7	7	5	7	22	5	8	8	5
6	F	F	F	F	M	M	F	M	M	M	M	F	F	F	M	F
7	P	T	P	T	T	P	P	P	T	P	P	T	P	P	T	P
8	E	L	L	NW	L	E	V	L	L	V	L	L	V	V	L	E
9	N	N	N	N	N	Y	N	N	N	Y	N	N	N	N	N	Y
10	Y	N	N	N	Y	N	N	N	Y	N	Y	N	N	N	N	N
11	Y/3	Y/5	Y/7	Y/7	Y/5	Y/7	Y/6	Y/7	Y/7	Y/5	Y/7	Y/5	Y/5	Y/8	Y/3	Y/5
12	Y	N	Y	N	Y	Y	N	N	N	N	Y	N	N	Y	N	Y
13	3	5	10	7	5	7	6	7	7	5	7	8	5	8	3	5
14	Y/3	N	Y/3	N	Y/4	Y/7	N	N	Y/2	N	N	N	N	N	Y/3	Y/5
15	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
16	19200	4500	14000	13000	20400	7200	9400	16000	7200	30000	10400	17000	24000	15300	5900	18000
17	0.46	0.2	0.2	0.2	0.3	0.46	O.3	O.2	O.42	0.2	0.23	0.3	0.2	0.34	0.2	0.2
18	1.2L	46000	1.6L	1.5L	1.2L	80000	94000	1.2L	66000	1.4L	1.4L	1.6L	90000	1.3L	78000	1.0L
19	-	+	-	-	-	+	-	+	-	-	-	+	-	+	+	-
20	N	N	N	N	N	Y	N	N	N	N	N	Y	N	Y	Y	N
21	B	B	CSF	CSF	CSF	B	B	B	B	B	B	B	B	U	B	B
22	NG	NG	NG	NG	NG	G	G	NG	NG	NG	NG	NG	NG	NG	NG	NG

1	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122
2	1830	1847	1857	1866	2624	1886	1890	1892	1893	1910	1930	1944	1946	1498	1902
3	NI	S	NI	NI	S	PS	S	S	NI	NI	S	NI	S	S	NI
4	I	O	I	I	I	I	O	O	I	I	I	I	O	O	I
5	6	10	7	5	15	21	8	15	10	5	26	5	8	10	7
6	M	F	M	F	F	M	F	F	M	M	M	F	M	F	M
7	P	T	P	T	P	P	P	T	P	P	T	P	T	T	P
8	V	L	L	NW	V	L	L	NW	L	V	NW	V	L	L	L
9	Y	N	N	N	N	N	N	N	N	Y	N	N	N	N	N
10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
11	Y/6	Y/4	Y/7	Y/5	Y/10	Y/7	Y/3	Y/5	N	Y/5	Y/7	Y/5	Y/3	Y/5	Y/7
12	N	N	N	N	N	N	N	N	N	Y	N	Y	N	N	N
13	6	4	7	5	10	21	3	5	10	5	26	5	3	5	7
14	Y/2	N	N	N	Y/5	N	N	N	N	N	N	N	Y/3	N	N
15	N	N	N	N	N	Y	N	N	N	N	N	N	N	N	N
16	16000	7200	30000	10400	17000	14500	12300	4600	22000	8100	7600	12000	18000	10400	17000
17	O.22	O.42	0.2	0.23	0.3	O.2	0.3	0.4	0.3	0.2	0.4	O.23	0.25	0.23	0.3
18	1.2L	66000	1.4L	1.4L	1.6L	1.6L	2.2L	60000	87000	1.1L	2.0L	1.4L	1.4L	1.4L	1.6L
19	+	+	-	+	+	-	+	+	+	-	+	+	+	+	+
20	Y	Y	Y	Y	Y	N	Y	Y	N	N	N	N	Y	Y	Y
21	B	B	B	B	CSF	B	B	B	B	B	B	B	B	B	U
22	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

1	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137
2	1967	1976	1980	3782	3698	1999	2000	2002	2028	2031	2032	2081	2110	2130	2134
3	S	S	NI	NI	NI	NI	S	NI	S	S	S	S	S	S	S
4	O	O	I	I	I	I	O	I	O	O	O	I	O	I	O
5	18	27	9	17	16	5	8	18	12	8	7	7	7	10	20
6	F	M	F	F	M	M	M	M	F	F	M	F	F	F	F
7	T	P	P	T	P	P	P	P	T	P	P	T	P	P	P
8	L	V	V	NW	L	V	L	L	NW	V	L	NW	V	L	L
9	N	N	Y	N	N	Y	N	N	N	N	N	N	N	N	N
10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
11	Y/7	Y/7	Y/5	Y/10	Y/6	Y/5	Y/3	Y/10	Y/7	Y/8	Y/3	Y/3	Y/7	Y/4	Y/7
12	N	N	N	N	Y	N	N	N	Y	N	N	N	N	N	N
13	8	8	9	17	16	5	3	18	8	8	3	7	7	4	8
14	N	N	N	N	N	Y/2	N	N	N	Y/5	N	N	N	N	N
15	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
16	12300	14000	16000	17200	30000	14000	13000	20400	4600	7200	12000	13000	14000	7200	23000
17	0.2	0.25	O.22	O.2	0.2	0.2	0.26	0.3	0.4	O.42	O.23	0.26	0.2	O.42	0.2
18	1.2L	2.0L	1.2L	1.6L	1.4L	1.6L	1.5L	1.2L	60000	66000	1.4L	1.5L	2.0L	66000	1.6L
19	+	+	+	-	-	-	-	-	+	+	+	-	+	-	+
20	Y	Y	N	N	N	N	N	N	Y	N	N	N	Y	N	Y
21	B	B	B	CSF	CSF	B	B	B	B	B	B	B	B	B	B
22	NG	NG	NG	NG	NG	NG	NG	NG	NG	G	NG	NG	NG	NG	NG

1	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152
2	2144	2145	2154	2170	2178	2200	2220	2225	2239	2266	2285	2289	2292	2294	2296
3	S	NI	NI	NI	S	S	S	PS	S	S	S	S	S	S	NI
4	O	I	I	I	O	O	O	O	O	O	O	I	O	O	I
5	12	12	25	10	16	28	10	38	18	34	17	10	24	20	11
6	F	F	M	M	F	F	M	M	M	F	M	F	F	F	M
7	T	P	P	P	P	P	P	T	T	P	P	P	T	P	T
8	L	V	L	L	V	L	V	NW	L	L	L	V	NW	L	NW
9	N	Y	N	N	N	N	Y	N	N	N	N	N	N	N	N
10	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N
11	Y/5	Y/7	Y/10	Y/5	Y/7	Y/8	Y/9	Y/20	Y/8	Y/8	Y/3	Y/10	Y/7	Y/4	Y/7
12	N	N	N	N	N	N	Y	N	N	N	N	N	N	N	N
13	5	12	25	10	8	8	9	20	8	8	3	10	8	4	11
14	N	Y/2	N	N	Y/2	N	Y/4	Y/5	N	N	N	N	Y/3	N	N
15	N	N	N	N	N	N	N	Y	N	N	N	N	N	N	N
16	12300	14000	20400	4600	7200	23000	4400	16000	4600	12000	13000	20400	7600	6500	8400
17	0.2	0.2	0.3	0.4	O.42	0.2	0.3	O.22	0.4	0.27	0.26	0.3	0.4	0.24	0.25
18	1.2L	2.0L	1.2L	60000	46000	1.6L	1.7L	1.2L	60000	2.6	1.5L	1.2L	2.0L	1.6L	1.8L
19	-	P	-	P	P	P	P	P	P	P	P	P	P	P	P
20	N	Y	N	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y	Y
21	B	B	B	B	B	B	U	B	B	B	B	B	B	B	B
22	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

1	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168
2	2311	2326	2343	2362	2392	2407	2483	2498	2505	2522	2524	2545	2564	2579	2580	2581
3	S	S	S	NI	S	S	NI	PS	S	S	NI	NI	NI	NI	NI	NI
4	O	O	I	I	O	O	I	O	O	O	I	I	I	I	I	I
5	22	28	18	28	16	6	5	34	12	5	5	8	5	8	8	9
6	M	F	M	F	F	M	F	F	M	F	M	F	M	F	M	F
7	T	T	T	PT	T	P	P	T	P	P	P	T	T	T	P	P
8	NW	L	NW	L	NW	L	L	NW	L	V	L	L	NW	L	L	V
9	N	N	N	N	N	N	N	N	N	Y	N	N	N	N	N	Y
10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
11	Y/8	Y/8	Y/3	Y/9	Y/5	Y/6	Y/5	Y/10	Y/6	Y/5	Y/3	Y/3	Y/5	Y/5	Y/3	Y/9
12	N	N	N	Y	N	N	N	N	Y	N	N	N	N	N	N	Y
13	8	8	3	28	5	6	5	10	6	5	5	8	5	8	8	9
14	N	N	N	Y/4	N	N	N	N	N	Y/2	N	N	N	N	N	Y/4
15	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
16	4600	12000	13000	4400	12300	14000	16000	17200	30000	14000	13000	20400	4600	10400	17000	4400
17	0.4	0.2	0.26	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.26	0.3	0.4	0.23	0.3	0.3
18	60000	2.6	1.5L	87000	1.2L	2.0L	1.2L	1.6L	1.4L	1.6L	1.5L	1.2L	60000	1.4L	1.6L	87000
19	+	+	+	+	+	-	+	-	-	-	-	-	+	+	-	+
20	Y	N	Y	Y	Y	N	N	N	N	N	N	N	Y	Y	N	Y
21	B	B	B	B	B	B	B	CSF	CSF	B	B	B	B	B	U	B
22	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	G

1	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184
2	2582	2583	2613	2635	2637	2640	2644	2666	2689	2690	2692	2693	2694	2719	2721	2752
3	S	NI	NI	S	NI	NI	S	NI	NI	NI	S	S	NI	S	NI	NI
4	O	I	I	O	I	I	O	I	I	I	O	O	I	O	I	I
5	5	8	6	20	7	5	10	7	7	5	6	8	5	5	8	8
6	M	F	M	M	M	M	F	M	F	M	F	M	M	M	F	M
7	P	T	P	P	P	P	T	P	P	P	P	T	T	T	P	P
8	L	L	L	V	V	L	L	L	V	L	L	N	L	L	L	V
9	N	N	N	N	N	N	N	N	N	Y	N	N	Y	Y	N	Y
10	Y	N	N	N	Y	N	Y	N	N	N	N	N	N	N	N	N
11	Y/5	Y/3	Y/6	Y/8	Y/7	Y/5	Y/5	Y/7	Y/7	Y/5	Y/3	Y/3	Y/5	Y/5	Y/3	Y/8
12	Y	N	N	N	Y	Y	Y	N	Y	N	N	N	Y	N	N	Y
13	5	8	6	8	7	5	5	7	7	5	3	3	5	5	8	8
14	N	N	Y/2	N	N	N	N	N	N	N	Y/3	N	N	Y/2	N	N
15	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
16	8100	13000	12000	17000	5500	8100	10400	17000	7200	16000	5900	9000	8100	14000	13000	15300
17	0.2	0.26	0.22	0.3	0.4	0.2	0.23	0.3	0.32	O.22	0.2	0.34	0.2	0.2	0.26	0.34
18	1.1L	1.5L	2.2L	1.6L	80000	1.1L	1.4L	1.6L	1.0L	1.2L	78000	1.2L	1.1L	1.6L	1.5L	1.3L
19	+	-	-	+	+	-	+	-	+	+	+	+	+	-	-	-
20	Y	N	N	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N	N	N
21	B	B	B	B	B	B	B	U	B	B	B	B	B	B	B	U
22	NG	NG	NG	NG	G	NG	NG	NG	G	NG	NG	G	NG	NG	NG	NG

1	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200
2	2753	2756	2767	2768	2770	2795	2797	2798	2820	2839	2867	2873	2943	2956	2966	2988
3	NI	NI	PS	S	NI	S	NI	S	S	S	NI	PS	S	NI	S	PS
4	I	I	O	O	I	O	O	O	O	O	I	O	O	I	O	I
5	35	5	16	5	8	16	5	10	20	8	16	35	15	22	15	10
6	M	M	F	F	M	F	M	M	M	M	M	M	M	M	M	M
7	P	P	T	P	T	T	P	P	T	T	T	T	P	T	P	T
8	V	L	L	V	L	NW	L	L	NW	NW	NW	L	V	L	L	L
9	N	N	N	Y	N	L	N	N	Y	N	N	N	N	N	N	N
10	N	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N
11	Y/10	Y/5	Y/7	Y/5	Y/7	Y/9	Y/5	Y/7	Y/5	Y/5	Y/7	Y/8	Y/3	Y/8	Y/3	Y/9
12	N	N	N	Y	N	Y	N	N	Y	N	N	N	N	N	N	Y
13	10	5	10	5	8	9	5	7	5	5	16	8	3	22	3	10
14	N	N	N	N	Y/3	Y/3	Y/3	N	N	N	N	N	N	N	N	Y/3
15	N	N	Y	N	N	Y/4	N	N	N	N	N	N	N	N	N	Y
16	20400	8100	17000	8100	13000	4400	12300	16000	8100	10400	17000	12000	13000	12000	13000	4400
17	0.3	0.2	0.2	0.2	0.26	0.3	0.2	0.3	0.2	0.23	0.3	0.27	0.26	0.27	0.26	0.3
18	1.2L	1.1L	1.6L	1.1L	1.5L	87000	1.2L	98000	1.1L	1.4L	1.6L	2.6	1.5L	2.6	1.5L	87000
19	-	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
20	N	Y	N	Y	N	Y	N	Y	Y	Y	Y	N	Y	N	Y	Y
21	U	B	B	B	B	B	B	B	B	B	U	B	B	B	B	B
22	NG	NG	NG	NG	NG	NG	NG	G	NG	NG	NG	NG	NG	NG	NG	NG

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Govt. Rajaji Hospital,
Madurai.20. Dated: 21.02.2014

Institutional Review Board / Independent Ethics Committee.
Captian. Dr. B. Santhakumar, M.D., (F.M.,)
Dean, Madurai Medical College &
Govt Rajaji Hospital, Madurai 625020. **Convenor**

Sub: Establishment-Govt. Rajaji Hospital, Madurai-20-
Ethics committee-Meeting Minutes- for January 2014
Approved list -regarding.

The Ethics Committee meeting of the Govt. Rajaji Hospital, Madurai was held on 20.1.2014, Monday at 10.00 am to 12.00.noon at the Anaesthesia Seminar Hall, Govt. Rajaji Hospital, Madurai. The following members of the committee have attended the meeting.

1.Dr. V. Nagarajan, M.D., D.M (Neuro) Ph: 0452-2629629 Cell.No 9843052029	Professor of Neurology (Retired) D.No.72, Vakkil New Street, Simmakkal, Madurai -1	Chairman
2. Dr.Mohan Prasad , M.S M.Ch Cell.No.9843050822 (Oncology)	Professor & H.O.D of Surgical Oncology(Retired) D.No.72, West Avani Moola Street, Madurai -1	Member Secretary
3. Dr. Parameswari M.D (Pharmacology) Cell.No.9994026056	Director of Pharmacology Madurai Medical College	Member
4. Dr.S. Vadivel Murugan, MD., (Gen.Medicine) Cell.No 9566543048	Professor of Medicine Madurai Medical College	Member
5. Dr.S. Meenakshi Sundaram, MS (Gen.Surgery) Cell.No 9842138031	Professor & H.O.D of Surgery Madurai Medical College	Member
6. Mrs. Mercy Immaculate Rubalatha, M.A., Med., Cell. No. 9367792650	50/5, Corporation Officer's quarters, Gandhi Museum Road, Thamukam, Madurai-20	Member
7. Thiru.Pala. Ramasamy , BA.,B.L., Cell.No 9842165127	Advocate, D.No.72.Palam Station Road, Sellur, Madurai -2	Member
8. Thiru. P.K.M. Chelliah ,B.A Cell.No 9894349599	Businessman, 21 Jawahar Street, Gandhi Nagar, Madurai-20	Member

17/3/14
To Dr L Lakshman Kumar
DIRECTOR,
INSTITUTE OF MICROBIOLOGY
MADURAI MEDICAL COLLEGE,
MADURAI - 625 020.

The following project was approved by the committee

..2..

Name of P.G.	Course	Name of the Project	Remarks
Dr.V. Lakshmana Kumar	PG in M.D., (Microbiology) Madurai Medical College and Government Rajaji Hospital, Madurai.	Characterisation and speciation of fungal isolates in neonatal sepsis in tertiary care centre.	Approved It should not be published, without written permission from Dean/Director of Medical Education.

Please note that the investigator should adhere the following: She/He should get a detailed informed consent from the patients/participants and maintain it Confidentially.

1. She/He should carry out the work without detrimental to regular activities as well as without extra expenditure to the institution or to Government.
2. She/He should inform the institution Ethical Committee, in case of any change of study procedure, site and investigation or guide.
3. She/He should not deviate the area of the work for which applied for Ethical clearance. She/He should inform the IEC immediately, in case of any adverse events or Serious adverse reactions.
4. She/He should abide to the rules and regulations of the institution.
5. She/He should complete the work within the specific period and if any Extension of time is required He/She should apply for permission again and do the work.
6. She/He should submit the summary of the work to the Ethical Committee on Completion of the work.
7. She/He should not claim any funds from the institution while doing the work or on completion.
8. She/He should understand that the members of IEC have the right to monitor the work with prior intimation.

Member Secretary **Chairman**
Ethical Committee

26.2.14
DEAN/Convenor
Govt. Rajaji Hospital,
Madurai- 20.

6/2/14

To
The above Applicant
-thro. Head of the Department concerned

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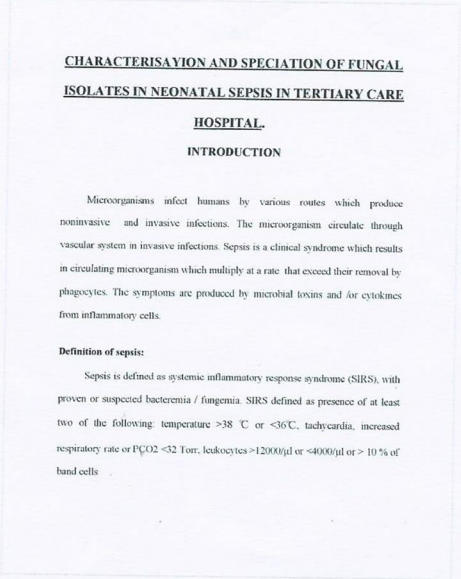


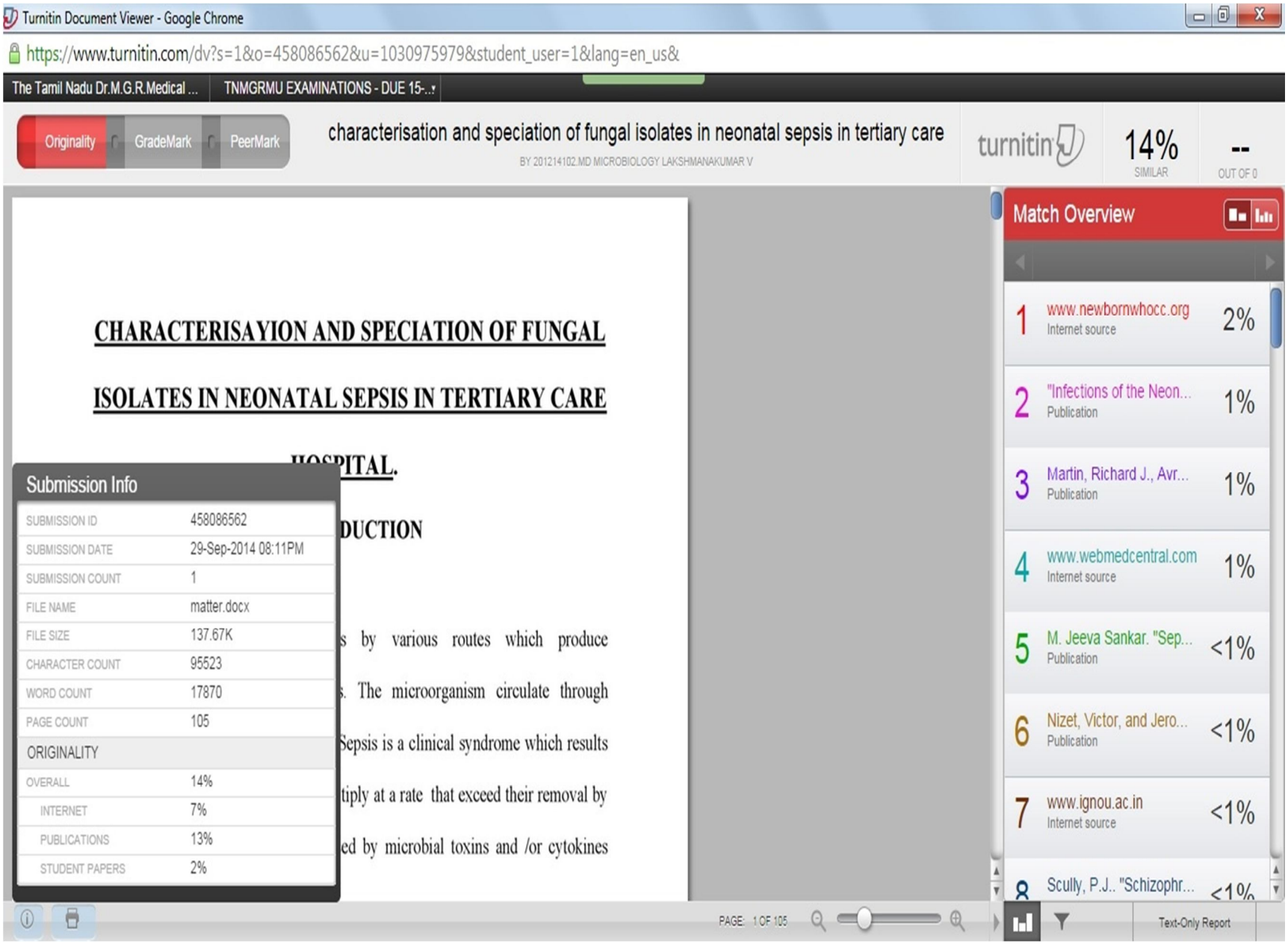
CHARACTERISAYION AND SPECIATION OF FUNGAL ISOLATES IN NEONATAL SEPSIS IN TERTIARY CARE HOSPITAL.

INTRODUCTION

Microorganisms infect humans by various routes which produce noninvasive and invasive infections. The microorganism circulate through vascular system in invasive infections. Sepsis is a clinical syndrome which results in circulating microorganism which multiply at a rate that exceed their removal by phagocytes. The symptoms are produced by microbial toxins and /or cytokines from inflammatory cells.

Definition of sepsis:

Sepsis is defined as systemic inflammatory response syndrome (SIRS), with proven or suspected bacteremia / fungemia. SIRS defined as presence of at least two of the following: temperature >38 ˚C or <36˚C, tachycardia, increased respiratory rate or PĊO2 <32 Torr; leukocytes >12000/µl or <4000/µl or > 10 % of band cells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HOSPITAL.
DUCTION
s by various routes which produce
s. The microorganism circulate through
Sepsis is a clinical syndrome which results
tiply at a rate that exceed their removal by
ed by microbial toxins and /or cytokines
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